DB Name	Query	Hit Count	Set Name
USPT,JPAB,EPAB,DWPI,TDBD	l10 and l11 and l12 and l14 and l17	11	L18
USPT,JPAB,EPAB,DWPI,TDBD	ampicillin	11779	L17
USPT,JPAB,EPAB,DWPI,TDBD	l10 and l11 and l12 and l14	16	L16
USPT,JPAB,EPAB,DWPI,TDBD	(phenylglycine amide) or (d-phenylglycine amide)	58	L15
USPT,JPAB,EPAB,DWPI,TDBD	(sulfuric acid) or h2so4	186645	L14
USPT,JPAB,EPAB,DWPI,TDBD	(sufuric acid) or h2so4	30723	L13
USPT,JPAB,EPAB,DWPI,TDBD	(d-phenylglycine) or phenylglycine	3390	L12
USPT,JPAB,EPAB,DWPI,TDBD	enzym$5	176652	L11
USPT,JPAB,EPAB,DWPI,TDBD	(6-aminopenicillanic acid) or 6-APA	1962	L10
JPAB,EPAB,DWPI	CN-1165032-$.did.	0	L9
JPAB,EPAB,DWPI	jp-05204120-$.did.	2	L8
JPAB,EPAB,DWPI	jp-02240026-$.did.	2	L7
JPAB,EPAB,DWPI	jp-05204120-$.did.	2	L6
JPAB,EPAB,DWPI	jp-5204120-$.did.	0	L5
JPAB,EPAB,DWPI	jp-2240026-$.did.	0	L4
JPAB,EPAB,DWPI	cn-1165032-$.did.	0	L3
JPAB,EPAB,DWPI	cn-1165032-$.did.	0	L2
JPAB,EPAB,DWPI	cn-1134306-$.did.	1	L1

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[illegible]

ABTI:
A [illegible] ester of the formula ##STR1## wherein R.sub.1 represents a hydrogen atom, a methyl group or an aryl group, and R.sub.2 represents a hydrogen atom or may be taken together with R.sub.1 to form a divalent benzo chain residue, or its acid addition salt.

ABTI:
The novel [illegible] ester or its acid addition salt is prepared by (1) reacting a reactive d-(-)-a-ylamino penicillanic acid (1) or its salt with a [illegible] of the formula ##STR2## wherein R.sub.1 and R.sub.2 are as defined above, and X is a re[illegible]-er atom, or reacting a compound of the formula ##STR3## wherein R.sub.1 and R.sub.2 are as defined above, or its acid addition salt with a corresponding carboxylic acid (1) or its reactive derivative, (2) thereafter, if required, when the resulting compound has the protected amino group or the group convertible to an amino group, deprotecting the protected amino group or converting said convertible group to an amino group, and (3) if further required, converting the product to an acid addition salt.

ABTI:
[illegible] also [illegible] an antibacterial agent comprising the novel [illegible] ester and a method for the treatment of infectious disease.

BSPR:
This invention relates to novel [illegible] esters, processes for their production, and to an antibacterial agent comprising such an [illegible] ester.

BSPR:
[illegible] which is [illegible] prepared by applying the acid group of [illegible] with .alpha.-aminophenylacetic acid is a [illegible] which is effective in oral administration. Absorption of [illegible] from the digestive tract, however, is not sufficient, and this [illegible] requires concentration in blood, which in turn causes increased side-effects.

BSPR:
[illegible] with a view to [illegible] to attain the above [illegible] of an ester-type derivative thereof improving its drawbacks in the [illegible], it is known, [illegible] of aminobenzyl penicillins [illegible] ampicillin, phthalidyl [illegible] ampicillin, and [illegible] ampicillin phthalidyl [illegible] ampicillin, and [illegible] ampicillin, etc. [illegible] comparable blood [illegible] ampicillin [illegible] in oral administration to those obtained by intramuscular administration.

BSPR:
It is an object of this invention to provide novel [illegible] esters [illegible] addition salts.

BSPR:
[illegible] to [illegible] and [illegible] antibacterial

[illegible] Ampicillin [illegible]

BSPR:
[illegible] Ampicillin esters [illegible] intestinal [illegible], have better absorption from the intestinal tract, maintain a high concentration in blood over longer periods of time and are less toxic than known Ampicillin esters such as Talampicillin.

BSPR:
[illegible]

BSPR:
A further object of this invention is to provide a novel process for production of the novel Ampicillin esters.

BSPR:
According to this aspect, these objects and advantages are achieved by Ampicillin esters of the general formula ##STR1## wherein R.sub.1 represents a hydrogen atom, a methyl group or an aryl group, and R.sub.2 represents a hydrogen atom, or may be taken together with R.sub.1 to form a divalent [illegible] chain [illegible], or their acid addition salts.

BSPR:
Specific examples of preferred Ampicillin esters of general formula (I) are:

BSPR:
The acid addition salts of these Ampicillin esters are, for example, salts of these esters with inorganic acids such as hydrochloric acid, hydrobromic acid, hydriodic acid and sulfuric acid, or salts of these with organic acids such as [illegible] acid and [illegible] acid.

BSPR:
Investigations of the present inventors have shown that the Ampicillin esters or their acid addition salts thereof have very desirable properties as pharmaceuticals.

BSPR:
Specifically, in oral administration, the Ampicillin esters of the invention are readily absorbed from the digestive tract, liberate Ampicillin in vivo, and maintain a high Ampicillin concentration in blood over long periods of time.

BSPR:
For example, thirty minutes after oral administration in mice, Ampicillin [illegible]-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride and Ampicillin [illegible]-oxo-5-phenyl-1,3-dioxolen-4-yl)methyl ester hydrochloride [illegible] Ampicillin concentration in blood about [illegible] times as high as that attained by the administration of Ampicillin and about 1.7 times as high as that attained by the administration of Ampicillin phthalidyl ester, and the high Ampicillin concentrations in blood are maintained over a long period of time. (See Experiment 1 given hereinbelow.

BSPR:
[illegible] Ampicillin esters of this invention [illegible] Ampicillin esters [illegible], they have [illegible]

BSPR:
[illegible] Ampicillin [illegible]-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester [illegible] Ampicillin [illegible]-oxo-5-phenyl-1,3-dioxolen-4-yl)methyl [illegible]

[illegible]

[illegible]:
[illegible]

[illegible]:
Experiments [illegible] are described below to demonstrate those advantages of the [illegible] of the invention.

[illegible]:
[illegible] Ampicillin. The blood was taken at the experimental animals periodically, and the concentration of Ampicillin in the serum was measured by a bioassay method. The blood Ampicillin level was calculated by the following equation. ##EQU1##

[illegible]:
The results given in Table 1 clearly show that the compounds of the invention show a high blood Ampicillin level over a longer period of time than the known [illegible].

[illegible]:
Prodrugs such as Ampicillin pivaloyloxymethyl ester or Ampicillin phthalidyl ester have been known as orally administrable Ampicillin. The ester group of the Ampicillin ester of the invention (i.e., 2-oxo-1,3-dioxolen-4-yl)methyl group) is shown by a formula below in comparison with those of the known prodrugs.

[illegible]:
It is clear therefore that the ester group of the Ampicillin ester of the invention quite differs from those of the known Ampicillin esters. It is surprising that the Ampicillin esters of the present invention have the aforesaid excellent properties as pharmaceuticals over those known Ampicillin esters.

[illegible]:
According to the process of the invention, the Ampicillin ester or its acid addition salt of the invention can be produced by reacting a compound of the general formula ##STR6## wherein A represents a protected amino group or a group convertible to an amino group, or its salt at the carboxyl group with a compound of the general formula ##STR7## wherein R.sub.1 and R.sub.2 are as defined above, and X represents a halogen atom, and if required, when the resulting compound has the protected amino group or the group convertible to an amino group, eliminating the protecting group from the protected amino group or converting the convertible group to an amino group, and if [illegible] required, converting the product to its acid addition salt.

[illegible]:
A compound corresponding to general formula II in which A is a free amino group is a compound well known as Ampicillin and readily available commercially.

[illegible]:
As a group, the [illegible] protected amino group in the group A in this case, the group A is desirably a protected amino group.

[illegible]:
The compound of general formula II can also be produced by reacting [illegible] or its salt at the carboxyl group with a carboxylic [illegible] ##STR11## wherein A is as defined hereinabove, or its reactive derivative at the carboxyl group. This is [illegible]

[illegible] of formula (II) are described, for example, in U.S. Pat. No. [illegible].

BSTR:
Thus, the Ampicillin ester of general formula (I) or its acid addition salt is formed. The acid addition salt is prepared by reacting the Ampicillin ester having a free amino group of general formula (I) with an acid, for example, an inorganic acid such as hydrochloric acid, hydrobromic acid, hydriodic acid and [illegible] or an organic acid such as citric acid or tartaric acid.

BSTR:
According to preferred embodiments of the process of the invention, there are provided a process for producing the Ampicillin ester of general formula (I) or its acid addition salt, which comprises reacting a compound of general formula (II) in which A is a [illegible] group or an amino group with the compound of general formula (III), and converting the [illegible] group or the [illegible] group of the product to an amino group, and thereafter if required, converting the product to its acid addition salt; and a process for producing a mineral acid salt (e.g., hydrochloride) of the Ampicillin ester of general formula (I) which comprises reacting a compound of general formula (II) in which A is an amino group in the form of a mineral acid such as hydrochloride, with the compound of general formula (III).

BSTR:
According to another process provided by the invention, the Ampicillin ester of general formula (I) or its acid addition salt can be produced by reacting a compound of general formula ##STR13## wherein R.sub.1 and R.sub.2 are as defined above, or its acid addition salt, with a carboxylic acid of the general formula ##STR14## wherein A is as defined above, or its reactive derivative at the carboxyl group; thereafter, if required, when the resulting compound has the protected amino group or the group convertible to an amino group, eliminating the protective group from the protected amino group, or converting said convertible group to an amino group, and if further required, converting the product to an acid addition salt thereof.

BSTR:
The compound of general formula (V) can be produced by reacting 6-aminopenicillanic acid or its salt at the carboxyl group with the compound of general formula (III); or by reacting 6-protected aminopenicillanic acid or its salt at the carboxyl group with the compound of general formula (III) and then converting the protected amino group of the reaction product to an amino group.

BSTR:
The former can be performed preferably by reacting 6-aminopenicillanic acid or its salt at the carboxyl group with an equimolar amount, or a molar excess, of the compound of general formula (III) in an inert organic solvent such as tetrahydrofuran, dioxane or acetone in the optional presence of a base (when 6-aminopenicillanic acid is used, the presence of a base is preferred) at a temperature of from about 0.degree. C. to room temperature.

BSPR:
The latter can be performed preferably by reacting 6-protected aminopenicillanic acid such as 6-aminopenicillanic acid having the amino group at the 6-position protected with an acyl group or trityl group, or 6-aminopenicillanic acid having the amino group at the 6-position protected as a Schiff base, or its salt at the carboxyl group, for example 6-phenylacetylaminopenicillanic acid (benzylpenicillin), with the compound of general formula (III) under the same conditions as in the first-mentioned process, thereafter reacting the resulting 6-protected aminopenicillanic acid ester with phosphorus pentachloride and a lower alcohol such as methanol at [illegible] in the presence of a basic compound such as N-methylmorpholine, pyridine and triethylamine, and thereafter causing water to [illegible] hydrolysis [illegible].

[illegible]

[illegible]

[illegible]

[illegible]

BSTR:
The Ampicillin ester of general formula (I) or its pharmaceutically acceptable acid addition salt is converted back to Ampicillin in vivo when administered to an animal. Accordingly, this invention also provides an antibacterial agent comprising the Ampicillin ester of general formula (I) or its pharmaceutically acceptable acid addition salt as an active ingredient.

BSTR:
The antibacterial agent of this invention may consist only of the Ampicillin ester of general formula (I) or its pharmaceutically acceptable acid addition salt, or a mixture of it with a pharmaceutically acceptable carrier.

BSTR:
The pharmaceutically acceptable carrier may be those carriers which can be used in formulating Ampicillin. Examples are starch, lactose, hydroxypropyl [illegible], hydroxypropyl cellulose, magnesium stearate, and calcium stearate.

BSTR:
The antibacterial agent of this invention is administered to man and other animals in a dose of 1 [illegible] mg/kg body weight/day calculated as the [illegible] of Ampicillin or a pharmaceutically acceptable salt.

BSTR:
Ampicillin 5-methyl-2-oxo-1,3-dioxolen-4-yl methyl ester R.sub.1 = methyl, R.sub.2 = hydrogen,

BSTR:
Ampicillin [illegible]-oxo-1,3-dioxolen-4-yl methyl ester R.sub.1 is R.sup.. = hydrogen,

BSTR:
Ampicillin 5-oxo-2-phenyl-1,3-dioxolen-4-yl methyl ester R.sub.1 = phenyl, R.sub.2 = hydrogen,

BSTR:
Ampicillin 5-oxo-2-[illegible]-1,3-dioxolen-4-yl ester R.sub.1 and R.sub.2 [illegible] -- R.sub.1 -- R.sub.2 , and

[0127]:

[illegible]

[0128]:
A. Ampicillin 5-methyl-2-oxo-1,3-dioxolen-4-yl methyl ester hydrochloride compounds of the invention.

[0129]:
B. [illegible]

[0130]:
C. [illegible]

[0131]:
TABLE 1 [illegible] Ampicillin level ratio [illegible] Compound 15 30 60 90 120 180 [illegible] Compounds A 2.1 2.9 2.1 1.5 1.1 1.1 at this interval, the [illegible] Known Compound C 1.3 1.8 1.4 1.1 1.9 1.1 Control Compound D 1.1 1.0 1.0 1.0 1.1 1.0

BSTL:

Ester group
Ampicillin pivaloyloxy methyl ester
##STR5## Ampicillin phthalidyl ester ##STR6## Ampicillin ester of the invention ##STR7##

[0132]:
[illegible] was dispersed in 6 ml of dimethyl formamide, and 140 mg of potassium hydrogen carbonate was added. The mixture was cooled [illegible]. To this solution, benzylbromide (0.06 ml) was added, and the mixture was stirred at 5.degree. C. for 2.5 hours. Then, 168 mg of potassium hydrogen carbonate and 212 mg of 4-bromomethyl-5-phenyl-1,3-dioxolen-2-one were added, and the mixture was further stirred at 5.degree. C. for 3 hours.

[0133]:
Water (5 ml) was added, and the mixture was concentrated under reduced pressure to distill off acetonitrile. The aqueous layer was repeatedly washed with ethyl acetate, and saturated with sodium chloride. The separated oily substance was extracted with 50 ml of methylene chloride, washed with a saturated aqueous solution of sodium chloride and dried over anhydrous sodium sulfate. The dried organic layer was concentrated until the amount of methylene chloride decreased to one half. Isopropyl alcohol (30 ml) was added, and the mixture was again concentrated under reduced pressure to give a colorless solid. The solid was collected by filtration, and washed successively with isopropyl alcohol and ether to give 320 mg (yield 46.4%) of [illegible] 5-methyl-2-oxo-1,3-dioxolen-4-yl methyl ester hydrochloride as a [illegible].

[0134]:
The resulting Ampicillin ester hydrochloride was incubated in 4% mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 15 minutes, and then subjected to high performance liquid chromatography. It was found to be completely converted to Ampicillin.

[0135]:
[illegible]

[illegible]

DEPP:
The solid was collected by filtration and washed with isopropyl alcohol and ether. [illegible] of Ampicillin
[illegible] ester by hydrochloride as a [illegible]

DEPP:
The resulting Ampicillin ester hydrochloride was incubated in 40% mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

DEPP:
By the same method as shown in Example 1, [illegible] mg of Ampicillin [illegible] was obtained as a [illegible] Ampicillin trihydrate and [illegible]

DEPP:
The resulting Ampicillin ester hydrochloride was incubated in 40% mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

DEPP:
Ampicillin trihydrate [illegible] was suspended in 4 ml of dimethyl formamide, and [illegible] was added. The mixture was [illegible] and 1 ml of benzaldehyde was added. The mixture was stirred at [illegible] C. for 3 hours. To the mixture were added 511 mg of potassium hydrogen carbonate and 1 g of 4-bromomethyl-5-methyl-2-oxo-1,3-dioxolene, and the mixture was stirred [illegible] hours.

DEPP:
Water [illegible] was added, and the mixture was concentrated under reduced pressure to remove acetonitrile. The aqueous layer was repeatedly washed with ethyl acetate, and saturated with sodium chloride to precipitate an oily substance. The aqueous layer was separated by decantation. The oily substance was dissolved in methanol, and treated with activated carbon, and [illegible] and the solvent was distilled off under reduced pressure to give a yellow syrup. The resulting syrupy product was dissolved in 4 ml of acetonitrile and the solution was adjusted to pH 2.0 with 10% hydrochloric acid. The solution was [illegible] ml of water, and the pH was adjusted to 6.0 with triethylamine. The aqueous layer was [illegible] and [illegible] with ethyl acetate. The aqueous layer was [illegible] and [illegible] ml of isopropyl alcohol was added, and the mixture was again concentrated under reduced pressure to give a colorless amorphous solid.

DEPP:
The solid was collected by filtration and washed with isopropyl alcohol and ether to give 511 mg of Ampicillin
[illegible] ester by hydrochloride as a [illegible] in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

DEPP:
By the same method as shown in Example 1, [illegible] mg of Ampicillin [illegible] was obtained as a [illegible] Ampicillin trihydrate and [illegible]

DEPP:
The resulting Ampicillin ester hydrochloride was incubated in 40% mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

DEPP:
Ampicillin trihydrate [illegible] was suspended in 4 ml of dimethyl formamide, and [illegible] was added. The mixture was [illegible] and 1 ml of benzaldehyde was added. The mixture was stirred at [illegible] C. for 3 hours. To the mixture were added 511 mg of potassium hydrogen carbonate and 1 g of 4-bromomethyl-5-methyl-2-oxo-1,3-dioxolene, and the mixture was stirred [illegible] hours.

DEPP:
Water [illegible] was added, and the mixture was concentrated under reduced pressure to remove acetonitrile. The aqueous layer was repeatedly washed with ethyl acetate, and saturated with sodium chloride to precipitate an oily substance. The aqueous layer was separated by decantation. The oily substance was dissolved in methanol, and treated with activated carbon, and [illegible] and the solvent was distilled off under reduced pressure to give a yellow syrup. The resulting syrupy product was dissolved in 4 ml of acetonitrile and the solution was adjusted to pH 2.0 with 10% hydrochloric acid. The solution was [illegible] ml of water, and the pH was adjusted to 6.0 with triethylamine. The aqueous layer was [illegible] and [illegible] with ethyl acetate. The aqueous layer was [illegible] and [illegible] ml of isopropyl alcohol was added, and the mixture was again concentrated under reduced pressure to give a colorless amorphous solid.

DEPP:
The solid was collected by filtration and washed with a mixture of water and methanol to give [illegible] mg of Ampicillin
(5-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride as a pale orange amorphous solid.

DEPP:
The resulting Ampicillin ester hydrochloride was incubated in 40% mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

[EEE]:

[FFF]:

[GGG]:
After the reaction, the solid was separated by filtration, and the filtrate was concentrated under reduced pressure. The resulting syrup was dissolved in water, and washed with ethyl acetate. The aqueous layer was saturated with sodium chloride, and the separated oily substance was extracted with methylene chloride. The extract was washed with a saturated aqueous solution of sodium chloride and concentrated until the amount of methylene chloride decreased to half. Upon addition of isopropyl alcohol, a colorless solid was precipitated. The solid was collected by filtration and washed with isopropyl alcohol and ether to give 132 mg (yield 54%) of Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride as an amorphous solid.

[HHH]:
From [illegible] mg of the [illegible] ester hydrochloride and 96 mg of D-(-)-phenylglycyl chloride hydrochloride, 148 mg (yield 56%) of Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride was obtained as a colorless amorphous solid.

[III]:
Five grams of (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl 6-aminopenicillanate-p-toluenesulfonate was suspended in 30 ml of ethyl acetate. To the suspension was added [illegible] ml of an aqueous solution of sodium hydrogen carbonate and [illegible] with [illegible]. The mixture was vigorously stirred. The ethyl acetate layer was separated, washed with ice water, dried at 5 degree. C. over anhydrous magnesium sulfate, and concentrated under reduced pressure to give a pale yellow syrup. The syrup was dissolved in 50 ml of methylene chloride. The solution was cooled to 5 degree. C., and 1 g of potassium hydrogen carbonate and 2.1 g of D-(-)-phenylglycyl chloride hydrochloride were added, and the mixture was stirred at 5 degree. C. for 4 hours. After the reaction, the insoluble material was separated by filtration, and the filtrate was concentrated under reduced pressure. The resulting syrup was dissolved in water and washed with ethyl acetate. The aqueous layer was saturated with sodium chloride. The separated oily substance was extracted with methylene chloride, washed with a saturated aqueous solution of sodium chloride and dried over anhydrous magnesium sulfate. The dried solution was concentrated under reduced pressure to about [illegible] ml, and [illegible] was added. The mixture was stirred [illegible] to give a [illegible]. The solid was collected by filtration, and washed with ether to give [illegible] mg (yield [illegible]%) of Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride as a colorless amorphous solid.

[JJJ]:
An ethanol solution of [illegible] hydroxypropyl cellulose was prepared and added to the [illegible] of water layer [illegible]. The mixture was stirred, [illegible] was distilled off, and the [illegible] was obtained.

.

[FTX]:
[illegible]

[TMPC]:
[illegible] Production of Amoxicillin (2-oxo-5-phenyl-1,3-dioxolen-4-yl) methyl ester [illegible]

[APC]:
[illegible] Preparation of Amoxicillin (2-oxo-5-phenyl-1,3-dioxolen-4-yl) methyl ester [illegible]

[BPC]:
[illegible] Preparation of Amoxicillin (2-oxo-5-phenyl-1,3-dioxolen-4-yl) methyl ester [illegible]

[DPC]:
Preparation of Amoxicillin (2-oxo-1,3-dioxolen-4-yl) methyl ester hydrochloride

[EPC]:
Production of Amoxicillin (2-oxo-5-phenyl-1,3-dioxolen-4-yl)methyl ester hydrochloride

[GPC]:
[illegible] of Amoxicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester [illegible]

[HTL]:
[illegible] (1) Amoxicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride 306.7 mg Lactose 38.3 mg Magnesium stearate 5.0 mg 400 mg in total [illegible]

[ITL]:
[illegible] (1) Amoxicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl) methyl ester hydrochloride [illegible] mg Lactose [illegible] mg [illegible]

[JTL]:
[illegible] (1) Amoxicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl) methyl ester hydrochloride 306.7 mg Crystalline cellulose 100 mg Lactose 28.3 mg Hydroxypropyl cellulose 10 mg Magnesium stearate 5 mg 450 mg in total

[KTB]:
1. An [illegible] represented by the formula [illegible] wherein R1 represents a hydrogen atom, a methyl group or an ethyl group, and R2 represents a hydrogen atom or a [illegible] which forms a [illegible] salt or which [illegible], or a pharmaceutically acceptable acid addition salt thereof.

[LTB]:
1. An antibacterial agent comprising an antibacterially effective amount of an Amoxicillin ester of the formula [illegible] wherein R1 represents a hydrogen atom, a methyl group or an ethyl group, and R2 represents a hydrogen atom or a [illegible] which forms a [illegible] salt or which [illegible], or a pharmaceutically acceptable acid addition salt thereof.

[illegible]:

[illegible]

[illegible]:

[illegible]

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[illegible]

BACKGROUND:
[illegible] .beta.-lactam derivatives with an [illegible] and
.beta.-lactam nucleus by acylation of the parent amine
.beta.-lactam with an acylating agent.

DESCRIPTION:
Today, semisynthetic .beta.-lactam derivatives such as ampicillin,
amoxicillin, cefaclor, cephalexin, cephradroxil and cephaloglycin are, in an
industrial scale, prepared by chemical methods, for example by reacting an
amino .beta.-lactam such as 6-aminopenicillanic acid, usually having its
carboxyl group protected, with an activated side chain derivative, followed by
the removal of the protecting group by hydrolysis. For example, ampicillin
(6-[D(-).alpha.-amino phenylacetamido-penicillanic acid) can be prepared by
reacting 6-APA, having a protected carboxyl group, with
D-phenylglycine [illegible], followed by hydrolysis of the protecting group by
mild acid. The reaction is typically [illegible] and at low
temperatures [illegible] and organic solvents like methylene chloride and
dimethyl formamide.

SUMMARY:
Within the last years, there has been an increasing amount of publications
concerning the possibility of enzymatic preparation of penicillins and
cephalosporins by [illegible] enzymatic acylation, i.e. by reacting the
.beta.-lactam nucleus, e.g. 6-APA, the parent amino .beta.-lactam, e.g. 6-APA, or
7-ADCA, the parent amino .beta.-lactam, e.g. 7-ADCA, with an activated
derivative of the side chain, e.g. an ester or amide thereof. For example,
the preparation of ampicillin from 6-APA and a D-phenyl-glycine derivative such as
a lower alkyl ester is known from West German patent application having
publication No. [illegible], Austrian Patent No. 243,[illegible], Dutch patent
application No. [illegible], West German patent application having publication
No. [illegible], European patent application having publication No. 339,[illegible] and
[illegible].

BRIEF:
In [illegible] with the present invention, an amino .beta.-lactam with an acylatable amino
group in the 6- or 7-position, i.e. 6-APA or 7-ADCA, is acylated by the
acylating .beta.-lactam derivative of the parent amino .beta.-lactam and
the .beta.-lactam nucleus having the same .beta.-lactam nucleus. In the
.beta.-lactam derivative which is the acylating agent, the [illegible] group
present in the parent .beta.-lactam nucleus is replaced by an acyl group
.beta.-lactam derivative.

DETAIL:
In the process according to the present invention, the .beta.-lactam derivative
which is the acylating agent, i.e. the .beta.-lactam derivative of the
.beta.-lactam nucleus having the same .beta.-lactam nucleus as the
.beta.-lactam derivative which is the acylating agent, is preferably an
activated ester or amide thereof. The [illegible] group
.beta.-lactam derivative formed may be due to hydrolysis thereof whereby
the .beta.-lactam acid form of the acylating agent is formed. In
addition, the [illegible] present may derive from the [illegible] acylating agent.

...hydrolytically, ...

BSPR:
In this way, particularly when fluid bed process conditions are employed in the enzymatic acylation of the amino .beta.-lactam is performed at least partly in the presence of the parent amino .beta.-lactam and the corresponding acylating agent. Preferably the concentration of the parent amino .beta.-lactam is maintained at a substantially constant level during the acylation, for example by the controlled addition of the parent amino .beta.-lactam during the acylation. Preferably the concentration of the parent amino .beta.-lactam is maintained at a level of from 1 to 10% of the saturation concentration of the corresponding acylating agent.

DEPR:
The .beta.-lactam derivative formed may precipitate during the reaction and, e.g., the acid addition salt of the acylating agent such as D-phenylglycine and D-p-hydroxyphenylglycine may precipitate. However, in some cases the reaction mixture remains a clear solution throughout.

PRIR:
The parent amino .beta.-lactam has a free amino group which is acylated by the reaction according to this invention. The amino .beta.-lactam may conveniently be 6-APA, 7-ADCA, 7-ACA or 7-ADAC.

DEPR:
The amino .beta.-lactam, for example 6-APA or 7-ADCA, used in the process of this invention may be obtained by enzymatic hydrolysis of the fermented penicillins or cephalosporins, for example penicillin V, penicillin G or cephalosporin C or their ring enlarged analogues (for example V-DCA and G-DCA or derivatives thereof followed by removal of the hydrolysis by-product, if desired (phenoxyacetic acid etc.). Advantageously, the crude solution can be used directly without further purification or dilution.

DEPR:
The acylating agent may be in an activated form. Preferably, the acylating agent is an amide or an ester. The acylating agent may be a derivative of D-phenylglycine, D-p-hydroxyphenylglycine, D-2,5-dihydrophenylglycine or mandelic acid, such as a lower alkyl ester (methyl, ethyl, n-propyl or isopropyl ester) or an amide which is unsubstituted or substituted in the ... group. The acylating agent may be used in the form of a salt, for example, the hydrochloride or the H.sub.2 SO.sub.4 salt. The acylating agent may be either in an active form or the active form may be formed in situ.

DEPR:
The solubility of the acylating agent such as the D-phenylglycine or D-p-hydroxyphenylglycine derivative will vary with the identity of the derivative and with the composition of the reaction medium. In an aqueous medium (as used in the examples), the solubility of the hydrochloride salt of D-phenylglycine amide is typically approximately 0.1 mM. However, the solubility is only dependent on the salt components in the solution, as well as on the pH value and the temperature of the solution. As a further example, the solubility of the sulphate form of D-phenylglycin amide is about 3.3 M within a pH range from 2.5 to 6.5.

DEPR:
Examples of .beta.-lactam derivatives that may be prepared by the process of this invention are the ... amoxicillin, cefaclor, cephalexin, cephradrine, cephalothin, cefazolin and cefadroxil.

DEPR:
The ... for use in the process of this invention may be any ... capable of acylating the amino group at the 6-position of penam or the 7-position of cephem nucleus. ... for use may, for example, be a penicillin acylase or a penicillin acylase and classified as E.C. 3.5.1.11. A number of microbial

[illegible] are known to have this activity, [illegible] for example [illegible] [illegible] the content of which is incorporated by reference.

DETD:

[illegible] the enzyme in a [illegible] form, for example, in [illegible] enzyme [illegible] commercially available from Boehringer Mannheim GmbH, Germany, under the trade name Enzygel.RTM., and [illegible] under the trade name Super Enzygel.RTM. Preferably, an enzyme [illegible], immobilized on a carrier consisting of a gelling polycarbohydrate and a polymer containing free amino groups, is applied.

DETD:

The suitable pH value depends, inter alia, on the type of the enzyme used. Using [illegible] enzyme, the pH value is typically in the range from [illegible] to [illegible], preferably in the range from [illegible] to [illegible]. For the preparation of amoxicillin, a pH value in the range from [illegible] to [illegible] is preferred. Control of the pH value may be used.

DETD:

Suitable enzyme concentrations may be from 1 to [illegible] U/ml (1 U = one unit of enzyme activity, see below).

DETD:

Using the process according to this invention, an extraordinary high molar ratio between the amount of [illegible]-lactam derivative which can be recovered and the total amount of side chain of the acylating agent can be obtained. [illegible] by the teaching of this invention and properly selecting the concentration of the acylating agent, the ratio between the concentration of acylating agent and the starting amino [illegible]-lactam, the pH value and the enzyme. Thus, a ratio of [illegible] was obtained in Example 1 below using the process according to the present invention. In a comparative batch process, vide Example 2 below, a molar ratio of only 1.4 was obtained. In addition, the yields of isolated product obtained in this example were [illegible] and [illegible], respectively.

DETD:

Herein, the following abbreviations have been used: 7-ADCA is [illegible]

DETD:

[illegible] is applied.

[illegible]:
[illegible]

[illegible]:
The equipment for this experiment consisted of one PFR, a thermostated reactor having a volume of 1.0 liter, equipped with a water-jacket thermostat and a pH-stat with a 1 M [illegible] for titrant. A valve was positioned at the outlet of the reactor. The outlet of the valve was connected via a pump to a basket centrifuge equipped with a polypropylene cloth having a porosity of 1-2 [illegible]. The outlet from the centrifuge was connected via a pump to a feed tank equipped with a stirrer and a glass sinter bottom. The outlet from the feed tank was connected via a pump to the reactor.

[illegible]:
A mixture of [illegible] (D-HPGM) (21.7 g, 120 mmol) and 6-APA (25.9 g, 120 mmol) in water was added to the reactor with the bottom valve closed. The stirrer was started. Immobilized benzylpenicillin G acylase (about 10 g, 5000 U/g) made up to 100 ml was added to the reactor. The pH value was maintained at 6.1. The reaction temperature was about 20 degree. C. Under these conditions, the reaction mixture was almost saturated with D-HPGM and 6-APA. Then, the bottom valve was opened allowing the reaction mixture from the reactor to enter the centrifuge. Thereafter, the mother liquor from the centrifuge was pumped into the feed tank wherein D-HPGM (21.7 g, 120 mmol) and 6-APA (25.9 g, 120 mmol) were loaded. The total volume of the suspension in the feed tank was kept at about 75 ml. A flow of about 100 ml/min was maintained in the system. The concentration of reaction components in the reactor, in the reactor outlet, in the centrifuge outlet, in the feed tank and in the feed tank outlet were monitored by analytical HPLC.

[illegible]:
As the reaction proceeded, the amoxicillin and D-HPG formed started to precipitate out of solution. The crystals were separated from the immobilized [illegible] particles by the bottom sieve in the reactor and the crystal suspension was [illegible] the centrifuge where the crystals were separated from the mother [illegible]. The mother liquor, which was then substantially free of [illegible], D-HPGM and 6-APA, passed into the feed tank, some of the solid D-HPGA and 6-APA present dissolved such that the outlet from the feed tank contained saturated D-HPGM and 6-APA. When the total concentrations of D-HPGM and 6-APA came down to about 225 mM HPGM and 225 mM 6-APA, more solid substrate was added to the feed tank. At intervals the crystals in the centrifuge were washed with water, the washing liquid was added to the reactor. The amount of water used was sufficient to keep the volume in the reactor at its starting [illegible]. After the separation, the centrifuge was emptied.

[illegible]:
After about 12 hours, the dosing of D-HPGM and 6-APA was stopped. After 14 hours, the 6-APA concentration reached 2 mM and the reaction was stopped. The amounts of reaction components are given in Table 1, below.

[illegible]:
A comparative experiment was performed in batchwise conditions and the reaction was stopped at the moment where the optimum yield of amoxicillin was obtained [illegible]. The reaction temperature was kept at 20 degrees. C., the pH was kept at 6.1 and the [illegible] of the enzyme, [illegible] in this example above, was used. The total volume of the reaction mixture was 1 liter. The [illegible] was put into the batch [illegible]. After 2 hours the [illegible] was [illegible], and the experiment was stopped with the [illegible] crystals [illegible] by the [illegible] in the reactor. The [illegible] was filtered. The amounts of reaction components are given in Table 2, below.

DEPR:
A mixture consisting of D-HPGA [illegible] g, [illegible] mmol) and 6-APA in [illegible] ml water, which was adjusted at a pH value of [illegible] by adding 4 M ammonium hydroxide, was added to the reactor with the bottom valve closed. The stirring was started. Immobilized Penicillin G acylase [illegible], size [illegible] made up to [illegible] ml was added to the reactor. The pH value was maintained at [illegible]. The reaction temperature was [illegible]. [illegible] these conditions the reaction mixture [illegible] with D-HPGA and 6-APA. Then the bottom valve was [illegible] the reaction mixture [illegible] the centrifuge. [illegible] the mother liquor [illegible] the centrifuge was pumped into the feed tank [illegible] D-HPGA [illegible] g, [illegible] and 6-APA [illegible] g, [illegible] mmol) were [illegible]. The total volume of the suspension in the feed tank was kept at about [illegible] ml. A flow of about [illegible] ml/min was maintained in the system. The concentration of reaction components in the reactor, in the reactor outlet, in the centrifuge outlet, in the feed tank, and in the feed tank outlet were [illegible] analyzed by HPLC.

DEPR:
The crystals formed in the reactor were separated from the immobilized [illegible] particles by the bottom sieve in the reactor. The crystal suspension was led to the centrifuge where the crystals were separated from the mother liquor. The mother liquor was passed through the feed tank. When the total concentrations in the feed tank came down to about 150 mM HPGA and 225 mM 6-APA, more solid was added to the feed tank. At intervals the crystals in the centrifuge were washed with water, the washing liquid was added to the reactor. The amount of water used was sufficient to keep the volume in the reactor at its starting level. After washing the crystals in the centrifuge, the centrifuge was emptied.

DEPR:
After about 12 hours, the dosing of 6-APA to the feed tank was stopped while the D-HPGA dosing was maintained in order to keep D-HPGA at saturation. After 14 hours, the 6-APA concentration reached 20 mM and the reaction was stopped. The amounts of reaction components are given in Table 3, below.

DEPL:
[illegible]

DETL:
HPLC Analysis of Ampicillin and Cephalexin

DETL:
Enzymatic Preparation of Amoxicillin from D-HPGM and 6-APA

DETL:
Enzymatic Preparation of Amoxycillin from D-HPGA and 6-APA

DETD:
Retention times in minutes: [illegible] D-PG ; [illegible] 7-ADCA ; [illegible] 6-APA ; [illegible] D-PGA ; [illegible] Cephalexin ; [illegible] Ampicillin ; [illegible] D-PGM.

DETD:
Retention times in minutes: 1.8 D-p-hydroxyphenylglycine ; [illegible] D-HPGM ; [illegible] D-HPGA ; [illegible] 6-APA ; [illegible] Amoxicillin.

DETL:
TABLE 3 [illegible] crystal cake in

[illegible] 6-APA [illegible] D-HPGA [illegible] mmol [illegible] Amoxicillin. 3H.sub.2 [illegible] D-HPG [illegible] mmol [illegible] mmol

Based on the above results, the [illegible] calculated amoxicillin yield (isolated product, based on 6-APA added): [illegible]. Amoxicillin productivity: [illegible] mmol/hour liter.

[illegible]

[illegible]

CLAIM:
14. The process according to claim 1, wherein the amino .beta.-lactam is selected from the group consisting of 6-aminopenicillanic acid, 7-amino-3-cephem-4-carboxylic acid, 7-aminocephalosporanic acid, 7-amino-3-acetoxymethyl-3-cephem-4-carboxylate and 7-amino-3-3-cephem-4-carboxylate.

CLAIM:
15. The process according to claim 1, wherein the acylating agent is selected from the group consisting of an activated form of D-phenylglycine, D-p-hydroxyphenylglycine, D-2,5-dihydrophenylglycine and mandelic acid.

CLAIM:
16. The process according claim 1, wherein the .beta.-lactam compound is selected from the group consisting of ampicillin, amoxicillin, cefaclor, cephalexin, cephadroxil, cephradine, epicillin and cefamandol.

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End of Result Set

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[illegible]

TITLE: Recovery of 1-phenyl-glycol- with [illegible] - by formation of pure, easily sepd. recyclable Schiff base, used in [illegible] prepn. of cephalexin, cefadrox, ampicillin, etc.

[illegible]

PATENT-ASSIGNEE:

ASSIGNEE	[illegible]
DSM NV	[illegible]

PRIORITY-DATA:

[illegible]	July 19, 1993

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	LABEL	MAIN-IPC
ES 2111251 T3	February 1, 1998	N/A	000	C12P035/04
WO 9503420 A1	February 2, 1995	E	012	C12P035/04
BE 1007296 A3	May 9, 1995	F	000	C12P000/00
EP 712443 A1	May 22, 1996	E	000	C12P035/04
[illegible]	[illegible]	N A	000	C12P035/04
[illegible]	[illegible]	F	000	[illegible]
[illegible]	[illegible]	N A	000	[illegible]

DESIGNATED-STATES: CN JP KR US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE AT BE ES FR GB IT NL PT AT DE ES FR GB IT NL PT

CITED-DOCUMENTS:EP 412594; EP 442591 ; US 4[illegible] ; [illegible] ; [illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

INT-CL (IPC): [illegible]; C12P 1/01; C12P 17/10; C12P 35/04; C12P 37/04

ABSTRACTED-PUB-NO: EP 712443B
BASIC-ABSTRACT:

[illegible] of a beta-lactam prod. is claimed in which a beta-lactam nucleus is [illegible] amide in an [illegible] reaction, and the enzyme, [illegible], and beta-lactam prod are sepd out. After the [illegible] reaction, the ppt. mixt. from which at least the enzyme and solid [illegible] have been removed, is treated with an aldehyde at a pH of [illegible] and the Schiff base of D-phenylglycine amide is sepd. out.

USE - The coupling reaction is used for prepn of important antibiotics, [illegible] ampicillin, cephalexin and cefadrin, in which the beta-lactam nucleus are prepd respectively from [illegible] , 7-amino-3-deacetoxy [illegible] , and 7-amino-3-chloro-4-carboxy-2-quinolone-8-thiol-8-oxide; the [illegible] with 7-ADCA, and [illegible].

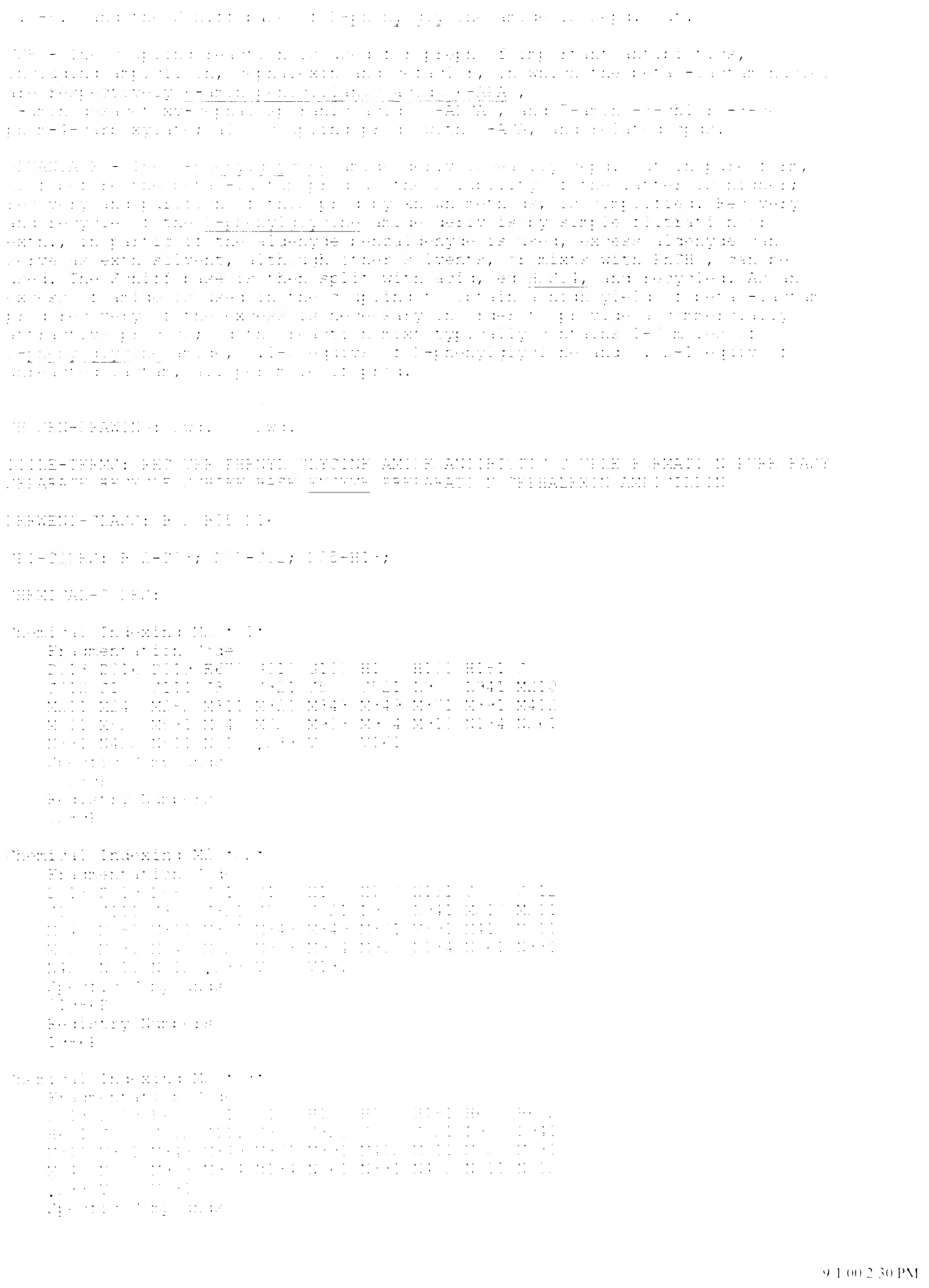
ADVANTAGE - The D-phenylglycine amide deriv is easily sepd. out in pure form, prior before the beta-lactam prod as the solubility of the latter is higher; recovery and purifiction of this prod by known methods, is simplified. Recovery and recycle of the D-phenylglycine amide deriv is by simple filtration or [illegible]. In particular the aldehyde benzaldehyde is used, excess aldehyde can serve as extn solvent, although other solvents, or mixts with PhOMe, can be used. The Schiff base is then split with acid, as HCl, and recycled. As an advantage it was found at the coupling to obtain a high yield of beta-lactam [illegible] by [illegible] enzyme be necessary to treat to provide a substantially [illegible]; [illegible] preferably for this 1-5 moles of D-phenylglycine amide, 1-5 equivs of D-phenylglycine and 1-5 equivs of [illegible].

ABSTRACTED-PUB-NO:

[illegible]

BASIC-ABSTRACT:

[illegible] - The beta-lactam prod. is claimed in which a beta-lactam nucleus is [illegible] amide, with an enzyme, and the enzyme, [illegible], and beta-lactam prod are sepd out. After the reaction, the ppt. mixt. from which at least the enzyme and solid [illegible] have been removed, is treated with an aldehyde at a pH of [illegible] and the Schiff base of D-phenylglycine amide is sepd. out.



UNLINKED-DERWENT-REGISTRY-NUMBERS: 0289P; 1986P

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L#: Entry # of 11 File: USPT [illegible]

DOCUMENT-IDENTIFIER: US [illegible] A
TITLE: Penicillin and cephalosporin intermediates

ABPL:
Novel penicillin and cephalosporin intermediates for the synthesis of 6-APA or 7-aminocephem compounds by the [illegible] formation of a [illegible] phosphite amide compound and the conversion of such compound to a desired 6-APA or 7-aminocephem compound.

BSPR:
Numerous methods for the synthesis of penicillins or cephalosporins have been described in the literature. The major part of these syntheses involves the reaction of 6-amino penicillanic acid (in the following referred to as 6-APA), salts and esters thereof or the corresponding 7-aminocephem compounds with an activated derivative of the acid, with which the amino group is to be acylated.

BSPR:
In one aspect of the invention there is provided a novel intermediate for the synthesis of derivatives of 6-APA or 7-aminocephem compounds in high yields.

BSPR:
The structure of the compounds having formula I has been established on the basis of their IR and NMR spectra [illegible]. The spin-spin coupling pattern of the latter shows that the 6.alpha.-hydrogen of the 6-APA derivative or the corresponding 7.alpha.-hydrogen of the 7-aminocephem derivative is strongly influenced by the introduction of the phosphorous atom.

BSPR:
In 6-APA derivatives with a free NH.sub.2 -group the proton in the 6-position is normally seen as a doublet at [illegible] ppm [illegible]. However, in an example of a 6-APA compound having the formula I, cf. Example 1, this proton is found as a multiplet consisting of [illegible] peaks in the region of [illegible] ppm [illegible], from which by first order analysis the following [illegible] coupling constants can be calculated:

BSPR:
It is even more surprising that a compound having the formula I, wherein R is a secondary ammonium group can be reacted with a halophosphite compound to form the corresponding phosphiteamide compound. However, it has been found that for example 6-aminopenicillanic acid or 7-aminodesacetoxycephalosporanic acid, [illegible] in the presence of two [illegible] triethylamine [illegible] with for example ethylene chlorophosphite [illegible] diethylammonium salt of 6-ethylenephosphite aminopenicillanic acid or 7-ethylenephosphite aminodesacetoxycephalosporanic acid and diethylammonium chloride. It might have been expected that ethylene chlorophosphite would react exclusively with the excessive mole of secondary amine.

BSPR:
[illegible] in the preparation of penicillins and cephalosporins [illegible] from 6-aminopenicillanic acid, 7-aminocephem [illegible], [illegible] is frequently used in the presence of a

hydrohalide acceptor.

BSPR:
[illegible] 6-APA [illegible] an excess of [illegible] triethylamine [illegible] of 6-APA. Thus, for the further acylation reaction it may be preferred to remove the excessive alkyl amine salt, on the other hand, a hydrohalide acceptor is necessary to promote the reaction.

BSPR:
[illegible] 6-APA [illegible] triethylamine [illegible] at this stage of the synthesis, 1 mole of trialkylsilylhalide, 1 mole of ethylene chlorophosphite or 1 mole of a reactive alkyl halide may be added to form the corresponding ester.

BSPR:
Among other activated acid derivatives which have been used in the acylation of the amino group of 6-APA or derivatives thereof are various forms of activated esters, e.g. p-nitrophenyl, cyanomethyl esters and the like.

BSPR:
Few syntheses of penicillin or cephalosporin derivatives are known in which a free carboxylic acid is reacted with a derivative of 6-APA or a 7-aminocephem compound.

BSPR:
The specification of British Patent No. 1.268.536 discloses that 6-isocyanato penicillanic acids may be prepared by reacting phosgene with a 6-APA ester. The 6-isocyanato penicillanic ester may then be reacted with a free acid to yield a penicillin derivative. However, this reaction can only be performed with moderate yields, and furthermore this known synthesis is not fully acceptable because of the use of phosgene which is poisonous and technically difficult to handle.

BSPR:
The above reaction can be illustrated by the following reaction scheme, in which a 6-APA derivative of the formula I is reacted with a carboxylic acid: ##STR[illegible]## in which R has the meaning defined above, and R.sup.3 is an organic group.

BSPR:
In a further embodiment of the method of the invention phthalide esters of [illegible] can be prepared in high yields from 6-aminopenicillanic acid or 7-aminocephem acids without [illegible] epimerization at C.sub.[illegible] or at C.sub.[illegible].

BSPR:
[illegible] millimoles) of 6-aminopenicillanic acid (6-APA) are dissolved in [illegible] ml acetonitrile by addition of [illegible] ml ([illegible] millimoles) of triethylamine, [illegible] -[illegible]° C. To this solution is added [illegible] After stirring for [illegible] hour, during which time the temperature of the reaction mixture raised to room temperature, the triethylammonium chloride formed ([illegible] g, [illegible] millimoles) is filtered off.

BSPR:
[illegible] millimoles) of 6-aminopenicillanic acid are dissolved in [illegible] ml of [illegible] by addition of [illegible] ml ([illegible] millimoles) of triethylamine. The [illegible] and [illegible] g ([illegible] millimoles) of [illegible] is added in portions under dry nitrogen. The mixture is

[illegible]

[illegible]:
[illegible] is added to [illegible] to precipitate the triethylammonium salt. The salt is filtered off. The filtrate is evaporated to a white wax which is very hygroscopic. The product is stable in cold, dry solution and when stored under dry nitrogen. The product may contain small amounts of unreacted [illegible], triethylammonium salt.

[illegible]:
[illegible] are added in 5 ml dry acetonitrile. [illegible] of triethylamine. The [illegible] are added dropwise under dry nitrogen and at a temperature of about -40.degree. C. Subsequently the solution is stirred for about 1 hour at room temperature.

BSPR:
1.03 ml (10 millimoles) of diethylamine are added to 1.08 g (5 millimoles) of [illegible] suspended in 10 ml dry acetonitrile. After stirring in a nitrogen atmosphere for 2 minutes, a clear solution is obtained.

[illegible]:
[illegible] are added 1.4 ml (10 millimoles) of triethylamine, and the mixture is stirred at room temperature until a clear solution is formed. The solution is cooled to -40.degree. C, and 1.40 ml of millimoles) of ethylenechlorophosphite dissolved in 4 ml of dry methylenechloride is added under dry nitrogen. The mixture is stirred for 15 minutes at -40.degree. C, 15 minutes at 0.degree. C and 15 minutes at room temperature, thereafter 1.0 ml (8.3 millimoles) of trimethylchlorosilane dissolved in 1 ml of methylenechloride is added. The mixture is stirred for 5 minutes at room temperature, and subsequently the precipitated triethylamine hydrochloride is filtered off. The solution is evaporated to form a hard, pale yellow oil.

BSTR:
1 g of [illegible] is suspended in 10 ml of dry methylene chloride under nitrogen. To this mixture are added 1.41 ml of dry triethylamine and 1.01 ml of trimethylchlorosilane. After stirring for 1 hour the reaction mixture is cooled to -40.degree. C, and a solution of 0.67 ml of [illegible] in 5 ml of dry methylene chloride is added [illegible].

BSTR:
[illegible] millimoles) of 2-[illegible]-propyldipropylphosphite, HCl are then [illegible] and [illegible] are added [illegible].

[illegible] is poured into water. The [illegible] phase is [illegible] covered with [illegible] ml methylene chloride. The pH-value of the water phase is adjusted at [illegible] with [illegible] and [illegible] is added. The [illegible] is stirred for [illegible] hours while [illegible] is maintained at [illegible]. Finally the [illegible] is [illegible] at [illegible]. The precipitated [illegible] is separated by filtration, washed with water and dried in an exsiccator.

EXPL:
4.33 g (21 millimoles) of D(-)-phenylglycylchloride, hydrochloride are added in portions over a period of 1 minute to a solution in methylene chloride of [illegible] millimoles of trimethylsilyl-[illegible]-hydroxyphosphiteamido-penicillanate [illegible] as prepared in Example [illegible] and without isolation [illegible] triethylamine (42 mM) at pH 7, at a temperature of 5 degree. The progress of the reaction is followed by penicillinase titration which after a period of 2 hours at 5 degree. C shows that a yield of above 70% has been obtained. At this time the reaction mixture is poured into 100 ml ice-water, and after stirring for 15 minutes the reaction mixture is filtered. The water phase is covered by 20 ml ethylacetate and the pH-value of the water phase is adjusted at 2 with a NaOH solution. Subsequently, the ampicillin formed is precipitated as a sparingly soluble salt with .beta.-naphthalenesulphonic acid while maintaining the pH-value at 2. The reaction mixture is left to stand for 1 hour at 5 degree. C and is subsequently filtered and the residue is washed with [illegible] ml H2O and ethylacetate. After drying in vacuum [illegible] white ampicillin .beta.-naphthalenesulphonic acid salt corresponding to 64.5 % of the theoretical amount are obtained. A high-voltage electrophoregram showed one spot with mobility identical to that of authentic ampicillin. The IR spectrum was identical to that of the .beta.-naphthalenesulphonic acid salt of authentic ampicillin.

EXPL:
[illegible] is dissolved in [illegible] ml dry methylene chloride and liberated from the hydrochloride by the addition of [illegible] ml (4 millimoles) of triethylamine, [illegible] ml ([illegible] millimoles) of trimethylchlorosilan are added dropwise and the reaction mixture is stirred for 1 hour at room temperature. At -5 degree. C [illegible] ml (1 millimoles) of ethylene chlorophosphite dissolved in 2 ml dry alcohol-free chloroform are added dropwise. The reaction mixture is stirred for 1 hour at 5 degree. C.

EXPL:
[illegible] are dissolved in [illegible] ml of dry methylene chloride and treated as described in Example [illegible]. A solution of [illegible] millimoles of triethylamine in [illegible] ml of dry methylene chloride is added during 10 minutes. Ampicillin titration 1 minutes after the addition is completed shows a penicillin yield of 68 %, while titration after one and two hours both shows 70 % yield.

EXPL:
4 millimoles of N-[illegible]-D-phenylglycyl-6-.alpha.-phenylglycyl-penicillanic-trimethylsilylester are prepared from [illegible] millimoles of N-[illegible]-D-phenylglycyl-6-.alpha.-hydroxybenzyl-penicillanic acid, 10 ml of chloroform, [illegible] ml [illegible] millimoles of triethylamine and [illegible] ml 4 millimoles of trimethylchlorosilan by stirring for 30 minutes are added to a solution of 4

[illegible]

[illegible] 4-hydroxy-3,5-dimethoxybenzyl- [illegible] -penicillanate prepared as described in Example [illegible], 1.0 g (2.1 millimoles) of 4-nitrophenylacetic acid, and the mixture is stirred at room temperature with dry air bubbling through. After a reaction period of 1 hours at room temperature the reaction mixture shows a penicillin yield of [illegible] by [illegible]. The reaction mixture is cooled to 0 degrees C, and 5 ml of pyridine are added followed by 25 ml of dimethylsulfoxide, whereafter the reaction mixture is poured into 500 ml of ice-cold 1 N NaCl solution and stirred for 30 minutes. Then 150 ml of ethylacetate are added and the pH adjusted to 2. After 30 minutes the phases are separated, and the water phase is extracted three times with ethylacetate. 25 ml of water are added to the combined organic phases, and the pH is adjusted to 7 with KOH. The water phase is separated, 150 ml of n-butanol added and the water removed by azeotropic vacuum destillation. The crystalline crude product thus precipitated is filtered off. Yield: 3.16 g (51%) of a purity of 76% determined by penicillinase titration. The white, crystalline product shows a characteristic absorption in the IR spectrum corresponding to that of the potassium salt of 4-hydroxybenzylpenicillin, and the NMR spectrum also shows signals which are characteristic of said compound.

Example:
[illegible] 4 millimoles of phenoxyacetic acid is added to a solution of 6-beta-(2,2,2-trichloroethyl-4-methyl-2-beta-ethylenephosphiteamido-3-hydroxy-3-methyl-7-oxo-4-thia-1-aza-bicyclo-[3.2.0]-heptane-2-carboxylate in acetonitrile prepared as described in Example 12, except for the filtration to remove the precipitate of triethylammonium chloride. The mixture is stirred for 16 hours at room temperature. The reaction mixture is then poured into ice-water and extracted three times with ethylacetate. The combined organic phases are washed three times with 1 N sulfuric acid and three times with saturated sodium hydrogen carbonate solution, dried over $MgSO_4$ and evaporated in vacuum to form an amorphous powder.

Example:
4.7 g of N-carbobenzoxy-6-alpha-phenylglycine in dry methylene chloride at room temperature are added to the product of 6.8 g of benzyl 6-beta-ethylenephosphiteamidopenicillanate of the reaction described in Example 1 under nitrogen. The mixture is stirred for 4 hours, evaporated to dryness, [illegible] ethylacetate and shaken with ice-water [illegible], [illegible] and finally with ice-water [illegible]. The solution is dried [illegible] and evaporated in vacuum to give [illegible].

Example:
[illegible] 4 millimoles of N-tert-butoxycarbonyl-6-alpha-phenylglycine are added to a solution of 6-beta-(2,2,2-trichloroethyl-4-methyl-2-beta-ethylenephosphiteamido-3-hydroxy-3-methyl-7-oxo-4-thia-1-aza-bicyclo-[3.2.0]-heptane-2-carboxylate prepared as described in Example 12, except for the filtration to remove the precipitate of triethylammonium chloride. The mixture is stirred for 16 hours at room temperature. The reaction mixture is then poured into ice-water and extracted three times with ethylacetate. The [illegible]

[illegible]

B4BH:
[illegible] are dissolved in [illegible] of acetonitrile by addition of [illegible] of triethylamine, and the solution formed is stirred for [illegible]. [illegible] of triphenylphosphite is [illegible]. The [illegible] is then [illegible]. The [illegible] is washed [illegible] and the [illegible] is [illegible].

B4B5:
A solution of the triethylammonium salt of 6-ethylene phosphite amidopenicillanic acid was prepared from [illegible] g ([illegible] millimoles) of 6-amino-penicillanic acid in the manner described in Example 1 and the triethylammonium chloride formed was removed by filtration.

B5F3:
The NMR spectrum ([sup.1]H.sub.1 CW) of a sample of the reaction solution showed that all 6-aminopenicillanic acid had been converted into the corresponding [illegible] and the following characteristic signals were obtained:

B5P1:
has the meaning defined above with a salt of 6-APA or of 7-aminocephem acid or a derivative of said acids.

B5P1:
The coupling constants J.sub.P-N-C-H, J.sub.H-N-C-H and J.sub.H-C-C-H are consistent with those found in literature for P-N-CH.sub.3 compounds are normally found J.sub.P-H = 8.5 - 25 Hz (Jakman and Sternhell: Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, vol. 5, p. 284 [illegible]). The coupling J.sub.H-C-C-H = 4.5 Hz is also found in the signal [illegible] from the proton in the 5-position in 6-APA. This pattern in conjunction with the signal of the 3-hydrogen and the infrared absorption frequencies of the carbonyl group in the 3-position which are consistent with those normally found in 6-APA derivatives, is a conclusive proof for the presence of the structural element: ##STR6##

B4F7:
[illegible]

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[illegible]

DOCUMENT-IDENTIFIER: [illegible]
TITLE: [illegible]

[illegible]:
[illegible] by acylating 6-APA or 7-A[illegible] and their derivatives with the hydrochloride of [illegible]-chloride and derivatives thereof having a substituted phenyl group, whereby the said acid choride is obtained by reacting the substituted phenylglycine with reagents like phosphorus pentachloride, thionyl chloride and phosgene. Although improved processes for the preparation of D-(-)-2-(p-hydroxyphenyl)-glycyl-chloride hydrochloride and the crystalline hemidioxane solvate thereof are known from British Pat. No. 1,466,637 and No. 1,460,916 the acylation of 6-APA or 7-A[illegible] or its 3-methyl modification with the above-mentioned acylating agent did not hitherto lead to results aimed at, mainly because either the product formed was too impure that further recovery of a product of the required quality hardly appeared to be possible, or the starting [illegible]-2-(p-hydroxyphenyl)-glycyl chloride hydrochloride of the required quality (purity) is only available for economically unattractive prices, if at all.

[illegible]:
A process for the preparation of amoxicillintrihydrate is also known from published German patent application DT [illegible] comprising the acylation of 6-APA, which has been previously silylated, with [illegible]-(p-hydroxyphenyl)glycylchloride hydrochloride and leading to yields, which [illegible] the present practical requirements.

[illegible]:
However, in the preparation of D-(-)-2-(p-hydroxyphenyl)-glycyl chloride hydrochloride according to the British Pat. No. 1,46[illegible] and British Pat. No. 1,466,6[illegible], phosgene is used in a relatively difficultly manageable process in which a solid is reacted with a gas. Such a process is extremely expensive in a number of countries with very stringent safety regulations, if indeed it may be applied at all. For the same reason, the process described in British Pat. Nos. [illegible] and 1,[illegible] disclosing the preparation of [illegible]-isocyanatopenicillanic acid and 7-isocyanatocephalosporanic acid derivatives from [illegible] of 6-APA or 7-ACA or its 3-methyl modifications with phosgene and [illegible]

[illegible]:
Less attractive features of this process are that the process is carried out [illegible], that solvents become mixed so that recovery thereof [illegible], and that, when adding the dimethylacetamide [illegible], 6-APA, 7-A[illegible] or 7-A[illegible]

[illegible]:
Furthermore, a number of patent applications and patents disclose preparation methods of .alpha.-aminoacyl-penicillanic acid derivatives by acylating 6-APA with mixed anhydrides derived from modified Dane salts of D-(-)-amino-(p-hydroxyphenl)-acetic acid, such as those described in German

BSPR:
[illegible] Although it is further known from a number of patent applications such as Japanese Patent application No. 49-114687 and No. 49-046892, British patents No. 1,367,342 and No. 1,382,255 and German patent applications Ser. No. 2,460,649 and No. 2,621,615, to prepare amoxicillin from 6-APA and p-hydroxy-phenylglycine or lower alkyl esters thereof by enzymatic acylation, [illegible] for these types are also unsatisfactory for the desired purpose in view of the yields obtained and in the presence of the acylating enzyme in the amoxicillin-containing solution obtained.

BSPR:
It has been found that the way in which the silylation is carried out is very important for the final yield, and the silylation is preferably carried out in dry methylene chloride containing 2 to 3 equivalents of a tertiary amine such as triethylamine and an equivalent amount of TMCS (about 2 equivalents for amoxicillin and cefatrizine and 3 equivalents for cefuroxime , in such a way that the pH, measured by a pH electrode is adjusted at the end of the [illegible] to a pH value between 6.7 and [illegible], for example a pH scale value between 6.7 and 7.1, preferably 6.9. The pH electrode is a Beckmann pH meter type 7411, and a Radiometer GK 2321 C electrode or an Inpold, so-called gold electrode, at a temperature between 15 degree. and 25 degree. C. Therefore, disilylation of 6-A. or 7-A. or 7-ADA is preferably carried out with practically balanced molar amounts of tri-lower alkyl halosilane, such as TMCS, and tertiary amine such as TEA .

BSPR:
[illegible] the silylation at a temperature between -15 degree. C. or lower and a temperature between -10 degree. and -5 degree. C. Therefore, disilylation of 6-APA, 7-ADA or 7-ADA derivatives thereof are added [illegible] rapidly with stirring as well as pressure so that a temperature of -15 degree. C. is reached, whereafter the reaction mixture is stirred at -10 degree. C. for 2 hours. An excess of the formula I compound is [illegible] a further 1.5 to 2 hours. An excess (1-1.5 mol) is in the nature of the [illegible] employed, the excess (1-1.5 mol) being in the nature of the substituent of the 7-position group of the cephalosporanic nucleus. For the preparation of amoxicillin and cefadroxil a small excess is sufficient. In the case of cefatrizine, having an acylatable amino group in the benzenoacetyl side, at least 2 moles of the compound of formula I will be necessary.

BSPR:
[illegible] It will be appreciated that one of the most important advantages of the [illegible] is that the silylation and subsequent acylation of the [illegible] can be carried out in the same solvent, i.e. methylene chloride, thus the silylation and the acylation [illegible] the product [illegible] without the [illegible] of a [illegible] will favorably influence the purity of the final product, the use of a

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

6-APA in 100 ml of dry methylene chloride, [illegible] mmoles of triethylamine and 42 mmoles of trimethylchlorosilane. The pH value measured with a Radiometer pH meter type TTT 2C and a Radiometer GK 2401C electrode was adjusted at [illegible] at the end of the silylation reaction.

DEPR:
In substantially the same manner as described in Example [illegible], amoxicillin trihydrate was obtained in a yield of [illegible] having a [illegible] measured purity of [illegible], [illegible] purity of [illegible], starting from [illegible] mmoles of potassium
D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate in [illegible] ml of methylisobutylketone and [illegible] ml of tetrahydrofuran, [illegible] ml of N-methylmorpholine, 44 mmoles of ethyl chloroformate in [illegible] ml of methyl isobutylketone, 40 mmoles of 6-APA in [illegible] ml of dry methylene chloride, [illegible] mmoles of triethylamine and 42 mmoles of trimethylchlorosilane.

DEPR:
[illegible] g of 6-aminopenicillanic acid were weighed into a 1 l reaction vessel and [illegible] ml of methylene chloride were added thereto. After addition of [illegible] g of bis(trimethylsilyl)-urea, the mixture was refluxed for about 2.5 hours and the mixture was then cooled to 20.degree. C. The "pH" reading, on the scale of a Radiometer pH meter type TTT 2C, connected with a Radiometer GK-2401C electrode, was 6.3.

DEPR:
In the same manner as described in Example 13, 44.3 g of amoxicillin trihydrate having a purity of 97.7% were obtained by reaction of [illegible]
D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate and 35 g of 6-aminopenicillanic acid, previously silylated with 71.5 g of bis(trimethylsilyl)acetamide instead of the bis(trimethylsilyl)urea.

DEPR:
b) Silylation of 6-APA

DEPR:
[illegible] g of 6-APA are stirred in [illegible] ml of methylene chloride. After addition of [illegible] ml of triethylamine at ambient temperature and under stirring, [illegible] ml of trimethylchlorosilane are added in about [illegible] minutes at a temperature of [illegible].degree.-[illegible].degree. C. After additional stirring for 1 hour the "pH" value is adjusted to a final value of 6.7 by the addition of 4.8 ml of trimethylchlorosilan. The mixture is cooled to -40.degree. C.

DEPR:
In the same manner as described in Examples 13-15, 55.3 g of amoxicillin trihydrate are obtained in a yield of [illegible] having a purity of [illegible] according to the hydroxylamine method measurement, a biologically measured purity of [illegible], a mercurimetrically measured purity of [illegible], and an optical rotation [.alpha.].sub.D.sup.20 of [illegible], starting from [illegible] g of potassium D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate in [illegible] ml of methylene chloride and [illegible] ml of N-methylpyrrolidone, [illegible] ml of N-methylmorpholine, [illegible] ml of methyl chloroformate, [illegible] g of 6-APA in [illegible] ml of methylene chloride, [illegible] ml of triethylamine and [illegible] ml of trimethylchlorosilane. The "pH" value measured with a Radiometer pH meter TTT 2C, and a Radiometer GK 2401C electrode was adjusted at 6.7 at the end of the silylation reaction, while the solution of the mixed anhydride as well as the solution of silylated 6-APA were both previously cooled to -40.degree. C. and reacted at [illegible] [illegible]

DEPR:
In the same manner as described in Examples 13-15, [illegible] g of amoxicillin trihydrate are obtained in a yield of [illegible] having a purity of [illegible] according to the hydroxylamine method measurement, a biologically measured purity of [illegible], a mercurimetrically measured purity of [illegible] and an optical rotation [.alpha.].sub.D.sup.20 of [illegible], starting from [illegible] g of potassium

[illegible]

[Blk]:
In the same manner as described in examples 10-16, amoxicillin was prepared in a 81% acylation yield starting from 88.1 g of potassium
D-.alpha.-[4-ethyl-2,3-dioxo-1-piperazinylcarbonylamino]-p-hydroxyphenylacetate in 471 ml of methylene chloride, 7.1 ml of N,N-dimethylformamide, 1.5 ml of N-methylmorpholine, 0.5 g of p-toluenesulfonic acid monohydrate, 27 g of 6-APA in 200 ml of water, 1.5 g of sodium bicarbonate and 27 ml of triethylamine. The "pH" was measured with a Beckman pH meter 3560, and a Parameter 3M pH electrode at 7.0 at the end of the silylation reaction, which was carried out at -4.degree. C. for 2 hours.

DEPC:
STEP B: Silylation of 6-APA

IBIL:
STEP B: Silylation of 6-APA

DEPC:
STEP B: Silylation of 6-APA

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[illegible]

[illegible]

[illegible]

[illegible] N,N'-carbonyldiimidazole or of an N,N'-carbonylditriazole [illegible] reagent, for example, N,N'-diisopropyl carbodiimide, N,N'-dicyclohexyl carbodiimide or N-cyclohexyl-N'-(2-morpholinoethyl)-carbodiimide.

[illegible]

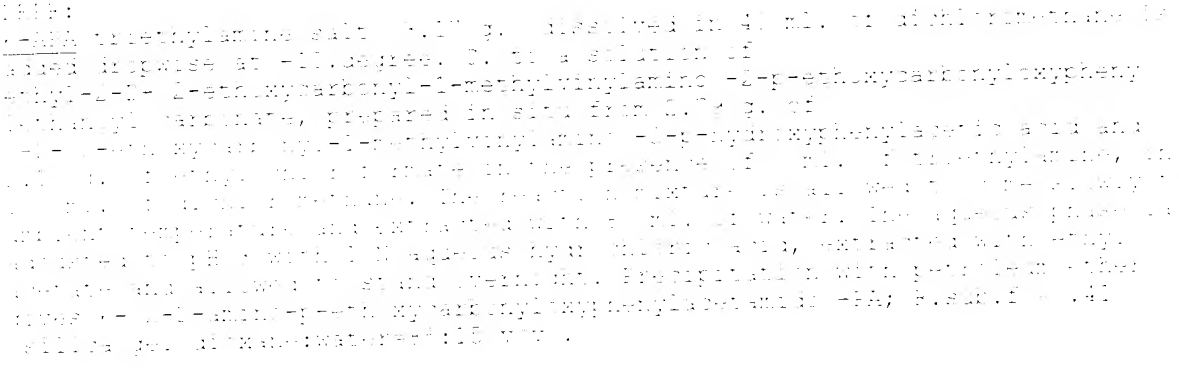
[illegible] is a N-carboxyanhydride (Leuchs' anhydride). The group which activates the carboxyl group here also protects the amino group.

[illegible] solvent, such as DMF or DMSO; or in a solvent mixture.

[illegible]

[illegible]

[illegible]



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[illegible] May [illegible]

[illegible]

ABPL:
[illegible] catalyzed acylation [illegible] the parent .beta.-lactam with an activated derivative of the side chain acid wherein a modulator, which consists of one or more compounds different from the reactants and the reaction product and which suppresses the hydrolysis of the activated derivative of the side chain acid and the desired product more than it suppresses the synthesis of the desired product, is added to the reaction mixture, at the beginning of the reaction process, in a concentration from about [illegible] to [illegible].times.10.sup.3 .mu.m.

BSPR:
The present invention relates to an improved method for enzymatic acylation. In particular, the invention relates to the preparation of .beta.-lactam antibiotics by enzymatic acylation of the parent amino .beta.-lactam moiety with an acylating agent which is an activated derivative of the side chain acid.

BSPR:
Enzymatic production of semisynthetic .beta.-lactam antibiotics by acylation of the parent amino .beta.-lactam moiety with the side chain acid or an activated derivative, such as an amide or an ester thereof, is known e.g. from West German patent application having publication No. [illegible], Austrian Patent No. [illegible], Dutch patent application No. [illegible], West German patent application having publication No. [illegible], European patent application having publication No. [illegible], international patent application having publication No. [illegible] and from international patent application having publication No. [illegible].

BSPR:
The parent amino .beta.-lactams such as 6-aminopenicillanic acid (6-APA) and 7-amino[illegible]cephalosporanic acid (7-ADCA) are commonly produced by [illegible] 6-APA or 7-ADCA. In the 7-ADCA case, the fermented penicillin has to [illegible] process before the hydrolysis step.

BSPR:
[illegible] enzymatic production of .beta.-lactam [illegible] with an activated [illegible] with the amino .beta.-lactam. Thus, when the amide of the side chain acid is used as acylating agent, some free side chain acid and an equivalent amount of [illegible] will be generated in the reaction mixture as a result of this hydrolysis. Similarly, when an ester of the side chain acid is used as acylating agent, some free side chain acid and an equivalent amount of the alcohol corresponding to the ester will be generated in the reaction mixture as a

[illegible] of the hydrolysis. Also, the desired product [illegible] hydrolysis [illegible]
free side chain acid and the parent amino .beta.-lactam.

BSPR:
It has now, surprisingly, been found that certain modulators, i.e. compounds different from the reactants and the reaction product, can be added to a reaction mixture in which enzymatic synthesis of a .beta.-lactam antibiotic [illegible], in a concentration which is lower than that of the reactants, preferably lower than about 100 mM, to suppress the reaction in such a way that the hydrolysis of the acylating agent--which is an activated derivative of the side chain acid is used as acylating agent--and of the desired product is suppressed more than the synthesis of the desired product.

BSPR:
Accordingly, in one embodiment aspect the present invention relates to a method of producing a .beta.-lactam antibiotic by enzymatic acylation of the parent amino .beta.-lactam with an activated derivative of the side chain acid wherein a modulator which suppresses the hydrolysis of the acylating agent and the desired product more than it suppresses the synthesis of the desired product is added to or present in the reaction mixture.

BSPR:
Examples of .beta.-lactam antibiotics which can be produced by the process of this invention are ampicillin, amoxicillin, ticarcillin, cefaclor, cefatrizine, cefaparol, cephradine, cephalexin, cefadroxil, cephaloglycin and cephalotin.

BSPR:
The acylating agent to be used in the method of this invention is an activated derivative of the side chain acid such as a lower alkyl (methyl, ethyl, n-propyl or isopropyl) ester or an amide. The amide can be unsubstituted in the --NH.sub.2 group which is preferred, or it can be substituted by one or two lower alkyl groups--identical or different--selected from the group comprising methyl, ethyl, propyl and isopropyl. The derivative may be used in the form of a salt, for example, the hydrochloride or the sulphate. Examples of side chain acids are D-phenylglycine or D-p-hydroxyphenylglycine.

BSPR:
Examples of parent amino .beta.-lactams which can be acylated by the method of this invention are 6-aminopenicillanic acid (6-APA), 7-aminocephalosporanic acid (7-ACA), 7-aminodesacetoxycephalosporanic acid (7-ADCA) and 7-amino-3-chloro-3-cephem-4-carboxylate.

BSPR:
The amount of modulator to be added to the reaction mixture in order to obtain the desired effect depends i.a. on the identity of the modulator and [illegible]. It is thus important to [illegible] in the examples and in the claims. [illegible] that a too high concentration of the modulator will prevent the desired reaction from taking place. Under all circumstances the useful concentration of modulator in the reaction mixture is so low that it does not influence the water activity in the reaction mixture. In any case, it will be lower than that of the reactants, preferably lower than 100 mM.

BSPR:
The enzyme to be used in the process of this invention may be any enzyme catalyzing the reaction in question. Such enzymes have been known since around [illegible], for example, [illegible] penicillin amidase [illegible] enzyme [illegible] Flavobacterium [illegible] patent application No. [illegible], Aphanocladium, Cephalosporium (West German patent application laying-open publication No. [illegible]), Acetobacter pasteurianum, Bacillus megaterium, [illegible]

[illegible]

[illegible]

[illegible]

DETD:
The amino .beta.-lactam, for example 6-APA or 7-ADCA, used in the process of this invention may be obtained by enzymatic hydrolysis of the fermented [illegible] penicillin V, penicillin G or [illegible] for example 6-APA and [illegible] hydrolysis [illegible] In some cases, the [illegible] with or further purification or dilution.

DETD:
Generally, the reaction temperature of the process of this invention may vary between about 0.degree. C. and about 80.degree. C., especially between about 5.degree. C. and about 45.degree. C. Temperatures in the range about 20.degree.-30.degree. C. may be preferred for convenient operation. The pH value may be optimal depends on the type and purity of enzyme. Using [illegible] the initial pH value is typically in the range [illegible] about [illegible], preferably in the range from about [illegible] about [illegible]. For the preparation of amoxicillin, a pH value in the range from about 8.5 to about 9.4 is preferred. Control of the pH value may be used. Suitable reaction times are from several minutes to several hours, in particular from about [illegible] hour to about 2 hours. Suitable enzyme concentrations may be from about [illegible] about [illegible] [illegible] activity, see [illegible].

[illegible]

[illegible]

[illegible], and [illegible] acid.

DEPR:
[illegible] Amox [illegible] D-HPG [illegible] enzyme, [illegible] enzyme activity.

DEPR:
In the following [illegible] penicillin G acylase activity is used: the unit [illegible] the amount of enzyme that hydrolyses per minute 1 [illegible] of penicillin G under standard conditions [illegible] penicillin G, [illegible] sodium phosphate buffer, pH [illegible], [illegible].degree. C).

DEPR:
The ratio X is defined as [illegible] D-HPG [illegible] per mole of Amox produced. For practical use this can be translated to: X=[illegible] Amox, wherein "[illegible] D-HPG" is the molar amount of D-HPG produced by hydrolysis of the acylating agent and the desired product and "moles Amox" is the molar amount of Amox present in the reaction mixture. Thus, if X is 0 this means that only the desired synthesis takes place, no hydrolysis. If X is 1, this means that D-HPG and Amox are formed in equal amounts (on a molar basis). If X is 2, this means that twice as much D-HPG as Amox is present in the reaction mixture (on a molar basis). The ratio X can be calculated at any time during reaction, but in the following examples X is calculated at the reaction stop time, which is defined as the time at which 90% of the theoretical yield of Amox is present in the reaction mixture (based on the inserted amount of 6-APA). Square brackets are used to designate molar concentrations.

DEPR:
Retention times in minutes: 2.6 (D-HPG), 3.5 (D-HPGA), 5.0 (6-APA), 13.5 (Amox).

DEPR:
In Examples 1-4 the following standard conditions for enzymatic amoxicillin synthesis have been used (see patent application No. WO [illegible] for further details):

DEPR:
During the reactions, the pH value of the reaction mixtures was kept constant by titration with [illegible].

DEPR:
A standard synthesis (immobilized penicillin G acylase from E. coli; enzyme dosing [illegible] U/ml) was carried out with no Phox added. The Phox level was [illegible] in the reaction mixture due to a residual Phox content of [illegible] in [illegible]. The results are reported in Table 1.

DEPR:
A standard synthesis (immobilized penicillin G acylase from E. coli; enzyme dosing [illegible] U/ml) was carried out with no Phgl added. The Phox level in the reaction mixture was [illegible]. The results are reported in Table 2.

DEPR:
Four different immobilized Pen G acylase preparations were used: a) immobilized penicillin G acylase from E. coli; enzyme dosing [illegible] U/ml; b) [illegible], dosing [illegible] U/ml; c) immobilizate obtained from [illegible], dosing [illegible] U/ml; and d) immobilizate obtained from Boehringer Mannheim (experimental preparation), dosing [illegible] U/ml.

DEPR:
[illegible] Pen G acylase preparations were obtained from [illegible], and from [illegible]. An enzyme dosage of [illegible] U/ml were employed in the two series. The results are reported in Table [illegible].

TITLE:
A [illegible] Synthesis [illegible] Using Immobilized Penicillin G Acylase to Vary the [illegible] Concentration

TITLE:
A [illegible] Synthesis [illegible] Immobilized Penicillin G Acylase, [illegible] Concentration

TITLE:
[illegible] Synthesis [illegible] Immobilized Penicillin G Acylase to Vary the Phox Concentration

DESC:
Enzyme Activity

DESC:
Enzymatic Synthesis of Amoxicillin Using a Fixed Dosage of Immobilized Pen G Acylase and Varying the Phox Concentration in the Reaction Mixture from 2.6 to [illegible] mM

DESC:
Enzymatic Synthesis of Amoxicillin Using a Fixed Dosage of Immobilized Pen G Acylase and Varying the Phox Concentration in the Reaction Mixture from [illegible] to [illegible] mM

DESC:
Enzymatic Synthesis of Amoxicillin Using Various Immobilized Preparations of Pen G Acylase and Varying the Phox Concentration in the Reaction Mixture from [illegible] to [illegible] mM

DESC:
Enzymatic Synthesis of Amoxicillin Using Varying Amounts of Enzyme and a Constant Concentration of Phox

DESC:
Enzymatic Synthesis of Amoxicillin by Using Soluble Pen G Acylase (Two Different Suppliers) and Varying the Phox Concentration in the Reaction Mixture from [illegible] to [illegible] mM

DESC:
[illegible] Synthesis of Amoxicillin by Using Immobilized Pen G Acylase and Varying the 6-Thienylacetic Acid Concentration from [illegible] to 0.1 mM

DESC:
Enzymatic Synthesis of Amoxicillin by Using Immobilized Pen G Acylase and Varying the 2-Thienylacetic Acid Concentration from 0.00 to 0.3 mM in the Reaction Mixture

DESC:
[illegible] Synthesis of Amoxicillin Using Immobilized Pen G Acylase and Various [illegible]

DESC:
TABLE 4

Initial Velocity (Vmax) [illegible] mM
Enzyme [illegible] Pen G Acylase Source Vmax (U/g) Km (mM) Vmax/Km
Experiment Number [illegible] Amox 6-APA Time

A 31 11.2 1.17 9.61 1.78 B 31 10.4 1.07

Title:

Abstract:

Title:
1. A method for preparing a semisynthetic beta-lactam antibiotic by the catalyzed hydrolysis of the parent beta-lactam with an amide or ester of the side chain acid wherein a modified, which is a carboxylic acid of a to 24 ... ester is derived from the reactants and the reaction product is ... with a mixture, at the termination of the reaction process, ...

Pub:
Japan. Kokai Patent, p. ... 1977.

Refs:
McDougall et al. Brumm Microb. Technol. 1982 vol. 4, pp. 114-116.

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[illegible] File: [illegible] [illegible]

[illegible]-IDENTIFIER: [illegible]
TITLE: [illegible] esters of penicillins

[illegible]:
[illegible] preferably salts with pharmaceutically acceptable inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, tartaric acid, citric acid, fumaric acid, and the like.

[illegible]:
In contrast to many of the corresponding free penicillins, the compounds of the invention are [illegible] absorbed from the gastrointestinal tract and are then, under the influence of enzymes, rapidly hydrolysed to the corresponding free penicillins. This hydrolysis is an important feature of the compounds of the invention. It is assumed that the first step consists in a hydrolysis to the hydroxymethyl esters of the corresponding penicillins which subsequently decompose spontaneously to the free penicillins.

[illegible]:
The compounds of the above formula VIIIa are new and can be prepared in different manners, for instance by reacting 6-aminopenicillanic acid with a [illegible] of the formula ##EXT## in which R.sub.[illegible], R.sub.[illegible], [illegible], and n are as [illegible] above. The reaction is preferably performed in the presence of an [illegible] amine, e.g. [illegible], and at room temperature or slightly elevated temperatures in an inert solvent, such as dimethylformamide, whereby an intermediate of the formula ##EXT## can be isolated which, after conversion of the group [illegible] into an unsubstituted or substituted amino group, yields the compounds of formula VIIIa. The conversion of [illegible] can be performed as described [illegible]. The amino group at the 6-position of the penicillanic acid can be, but is not necessarily, protected by well-known protecting groups, [illegible] [illegible].

[illegible]:
[illegible] Amino Acid R.sub.[illegible] M.p. [illegible] [illegible] 1. L-valine CH.sub.[illegible] [illegible] [illegible] 2. L-valine -- [illegible] -- 3. D,L-valine -- [illegible] -- 4. L-leucine [illegible] 5. L-isoleucine C.sub.2 H.sub.5 [illegible] 6. D,L-tert.leucine CH.sub.3 [illegible] .alpha.-methyl ester [illegible] 7. L-aspartic acid --[illegible] .beta.-methyl ester [illegible] 8. L-aspartic acid [illegible] 9. D-.alpha.-phenylglycine C.sub.6 H.sub.5 -- [illegible] [illegible] [illegible]

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[illegible]

ABTL:
Process for the recovery of ampicillin from a mixture containing ampicillin and 6-aminopenicillic acid (6-APA), in which a mixture of ampicillin and 6-APA, with a pH higher than [illegible], which apart from any solid ampicillin being present is homogeneous at a pH between [illegible] and 8.5, is subjected to a pH lowering till a pH lower than 6.2 is reached, and the solid substance present is removed. The process is in particular suitable to be applied to the [illegible] mixture which is obtained after the enzymatic acylation reaction of 6-APA with a phenylglycine derivative as acylating agent. Pure ampicillin can thus be recovered in a simple way.

BSPR:
The invention relates to a process for the recovery of ampicillin from a mixture containing ampicillin and 6-aminopenicillic acid (6-APA).

BSPR:
In the preparation of ampicillin, with 6-APA being acylated with a [illegible] derivative, the recovery of the ampicillin and washing out of the [illegible] are difficult in practice.

BSPR:
A process for isolating the ampicillin pure from a mixture containing ampicillin and minor quantities of 6-APA is described in JP-A-47030685. According to the process described in this Japanese publication, an acid aqueous mixture containing 6-APA and ampicillin is subjected to an extraction with butanol or isoamylalcohol, after which the pH is raised to a value between 4 and 5 and the product is recovered by complete boiling down and freeze-drying. The drawback of this method is that organic solvents that are [illegible] to be used. In addition, complete boiling down and freeze-drying is not industrially practicable. Moreover, the process involves a [illegible] of [illegible] in the freeze-dried product.

BSPR:
JP-A-[illegible] discloses a process wherein ampicillin is recovered from a mixture of ampicillin and 6-aminopenicillanic acid by conversion of the ampicillin to the trihydrate salt and isolation of the ampicillin as its trihydrate salt.

BSPR:
[illegible] the invention is to provide a simple, industrially practicable process for the recovery of ampicillin from a mixture of ampicillin and 6-APA, in which [illegible] and organic solvents that are difficult to remove from the product are avoided.

BSPR:
This is achieved according to the invention in that a mixture containing ampicillin and 6-APA and having a pH higher than [illegible], which, apart from any solid ampicillin that is present, is homogeneous at a pH between [illegible] and 8.5, is subjected to a pH lowering to a pH lower than 6.2, and that the solid substance present is removed.

BSPR:

[illegible]

the mixture, after which it can be recovered. Besides ampicillin the reaction mixture often contains other valuable components, such as for instance the [illegible] 6-APA. In order to get a commercially attractive process it is consequently [illegible] 6-APA and ampicillin [illegible] [illegible] the pH is lowered further to a value of less than [illegible] 6-APA and the remaining ampicillin [illegible] 6-APA and [illegible]

BSUB:
The process according to the invention is in particular suitable to be applied in the working up of the reaction mixture which is obtained after the enzymatic acylation reaction in which 6-APA is acylated with D-phenylglycine [illegible] D-phenylglycine. Thus, the process according to the invention can for instance be applied to a starting mixture that is obtained by successively filtering, with isolation of immobilized enzyme, the reaction mixture of an enzymatic acylation reaction carried out at a relatively high pH, for instance a pH between [illegible] and 10, in particular between 8 and 10, lowering the pH to a value between 7 and 9. Depending on the amount of D-phenylglycine (PG) formed during the acylation reaction, if desired, it is possible to remove first at a higher pH-value between 7 and 9--which is selected dependent on the mixture such that already PG has been crystallized out and ampicillin has not yet--the eventually formed solid substance, which mostly will consist mainly of PG.

BSUB:
[illegible] the mixture obtained after an enzymatic acylation reaction that ends at a relatively low pH, for instance a pH between [illegible], preferably between [illegible] and [illegible], and after isolation of the solid substance which mainly contains the immobilized enzyme and D-phenylglycine.

BSUB:
[illegible]

BSUB:
The pH may be lowered in several ways in the framework of the invention, for instance chemically by adding an acid, for instance a mineral acid, in particular sulphuric acid, hydrochloric acid or nitric acid. Another possibility is for instance, if PGA has been used as acylation agent in the [illegible] steam [illegible] thin-film evaporation; [illegible] electrodialysis.

BSUB:
[illegible] ampicillin [illegible] 6-APA and ampicillin [illegible] ampicillin [illegible] ampicillin [illegible] 6-APA [illegible]

[illegible], which [illegible] the pH at which the [illegible] optimum pH in a given situation.

BSTR:
The process according to the invention for recovery of pure ampicillin in [illegible] 7-ADA and ampicillin [illegible] enzymatic [illegible] 7-ADA [illegible], [illegible], may [illegible] pH higher than [illegible], in particular between [illegible] and [illegible]. If desired, it is possible to apply further concentration and cooling to a temperature lower than [illegible].degree. C., for instance between [illegible] and [illegible].degree. C. In this way a process is obtained with which both 7-ADA and [illegible] can be applied with a high efficiency. In the enzymatic acylation reaction, PGA or esters of PGA for instance may be used as acylation agent.

BSTR:
In principle any enzyme can be used that is suitable as catalyst in the [illegible] enzymes are for instance the enzymes that are known under the general designations 'penicillin amidase' and 'penicillin acylase'. Examples of suitable enzymes are enzymes derived from Acetobacter, Aeromonas, Alcaligenes, Alcaligenes, Aphanocladium, Bacillus sp., Cephalosporium, Escherichia, Flavobacterium, Kluyvera, Mycoplana, Protaminobacter, Pseudomonas and Xanthomonas, in particular Acetobacter pasteurianum, Bacillus megaterium, Escherichia coli and Xanthomonas citrii.

BSTR:
Preferably an immobilized enzyme is used, since the enzyme can be easily isolated and re-used then. Immobilized enzymes are known as such and are commercially available. Example of suitable enzymes are the Escherichia coli enzyme from Boehringer Mannheim GmbH, which is commercially available under the name 'Enzygel.RTM.', the immobilized Penicillin-G acylase from Recordati, the immobilized Penicillin-G acylase from Pharma Biotechnology Hannover, and an Escherichia coli penicilline acylase isolated as described in WO-A-[illegible] and immobilised as described in EP-A-[illegible].

BSTR:
The enzymatic acylation reaction is mostly carried out at a temperature lower than [illegible].degree. C., preferably between [illegible] and [illegible].degree. C. The pH at which the enzymatic acylation reaction is carried out is mostly between [illegible] and [illegible], preferably between [illegible] and [illegible].

BSTR:
In practice the enzymatic acylation reaction and the working up of the reaction mixture are mostly carried out in water. Optionally, the reaction mixture may also contain an organic solvent or a mixture of organic solvents, preferably less than [illegible] vol. [illegible]. Examples of organic solvents that can be used are alcohols with 1-7 carbon atoms, for instance a monoalcohol, in particular [illegible], in particular [illegible], in [illegible].

[illegible]:
AMPI=ampicillin

BSTY:
AMPI.3H.sub.2 O=ampicillin trihydrate

[illegible]:
[illegible]

[illegible]:

[illegible]

[illegible]:
[illegible]

[illegible]:
Enzymatic acylation of 217 mM of PGA and 217 mM of 7-ADCA at 5.degree. C., followed by acidifying up.

[illegible]:
A mixture of [illegible] g of 7-ADCA and [illegible] g of PGA was suspended in [illegible] ml of water and cooled to 5.degree. C. The resulting suspension was added to [illegible] g of "wet" immobilized Pen-G acylase from Recordati "Milan". This enzyme is commercially available in a mixture of water and glycerol ("wet enzyme"); before use it was washed three times with 100 ml of water.

[illegible]:
After 2 hours the pH was brought to 7.5 by means of concentrated aqueous NH.sub.3 the pH was brought to 8.6 and after 1 minutes the reaction mixture was filtered through a G-3 glass filter; the residue was washed with 100 ml of water (5.degree. C.). This residue was a mixture of enzyme and PG formed during the reaction.

[illegible]:
Enzymatic acylation of 217 mM of PGA and 217 mM of 7-ADCA at 5.degree. C., followed by acidifying up.

DEPR:
A mixture of 43.9 g of 6-APA and 30.6 g of PGA was suspended in 877 ml of water and cooled to 5.degree. C. The resulting suspension was added to 100 g of "wet" immobilized Pen-G acylase from Recordati "Milan". This enzyme is commercially available in a mixture of water and glycerol ("wet enzyme"); before use it was washed three times with 100 ml of water.

[illegible]:
After 2 hours the pH was brought to 7.5 by means of concentrated aqueous NH.sub.3 the pH was brought to 8.6 and after 1 minutes the reaction mixture was filtered through a G-3 glass filter; the residue was washed with 100 ml of water (5.degree. C.). This residue was a mixture of enzyme and PG formed during the reaction.

[illegible]:
[illegible]

[illegible]:
[illegible]

[illegible]:
[illegible]

[illegible]:
[illegible]

[illegible]:
[illegible]

[illegible]:
[illegible]

[illegible]:
[illegible]

"(ii)":
[illegible]

"(iii)":
[illegible]

"(iv)":
[illegible]

"(v)":
obtaining a mixture containing amphicillin and 6-amino penicillin that is prepared from the reaction mixture of an enzymatic acylation reaction in which 6-APA is acylated using D-phenylglycinamide (PGA) or esters of D-phenylglycine, said mixture having an initial pH greater than 7; and

"(vi)":
[illegible]

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L--: Entry [illegible] Date: 7/31 [illegible]

[illegible]-Publication: [illegible]
Title: Process for preparing .beta.-lactams through a chemical
transformation of acylating agent plus .beta.-lactam derivative at least 4[illegible]
mM

ABPR:
.beta.-lactam derivatives are synthesized by an enzymatic reaction of the
parent amino .beta.-lactam with the corresponding acylating agent, the
activation of the acylating agent plus the concentration of .beta.-lactam
derivative to at least 4[illegible] mM.

BSTR:
This invention relates to a process for the preparation of .beta.-lactam
derivatives by enzymatic acylation of the parent amino .beta.-lactam with an
acylating agent. The amino .beta.-lactam may be 6-aminopenicillanic acid
(6-APA), 7-aminodesacetoxycephalosporanic acid (7-ADCA),
7-aminocephalosporanic acid (7-ACA) or 7-amino-3-chloro-3-cephem-4-carboxylate
and the acylating agent may be a derivative of D-phenylglycine or
D-p-hydroxyphenylglycine.

BSTR:
Today, semisynthetic .beta.-lactams such as Ampicillin, Amoxicillin, Cefaclor,
Cephalexin, Cephadroxil and Cephaloglycin are prepared in industry by chemical
methods, for example by reacting an amino .beta.-lactam such as
6-aminopenicillanic acid, usually having its carboxyl group protected, with an
activated side chain derivative, followed by the removal of the protecting
group by hydrolysis. It is important due to, for example, yield, that the
amino .beta.-lactam, for example 6-APA, is used in a pure, dry form,
preferably in a purity higher than 97%. For example, Ampicillin
(D-(-)-.alpha.-amino-benzylpenicillanic acid) can be prepared by
reacting 6-APA, which is protected on the carboxyl group, with
D-(-)-mandelic chloride, followed by removal of the protecting group by
hydrolysis. These reactions typically involve costly steps such as sub zero
reaction conditions and organic solvents like methylene chloride and
[illegible].

BSTR:
[illegible] process for [illegible] from the 6-APA and a D-phenylglycine
derivative, such as a [illegible] ester, is known from West German patent
[illegible], Belgian Patent No. [illegible], Dutch patent application No. [illegible], Japanese
patent application No. [illegible], and European patent application publication No.
[illegible]. The [illegible] process disclosed in the prior art have typically used a low
pH of the [illegible] reaction medium of 6.5 or [illegible], the highest
pH mentioned was [illegible] European patent application having publication No.
[illegible].

BSTR:
The amino .beta.-lactam is [illegible] by [illegible] by [illegible]

[illegible] purification and crystallization [illegible] 6-APA or 7-ADCA [illegible], the [illegible] penicillin [illegible] in the hydrolysis step [illegible].

BSTB:
The potential drawbacks of the known enzymatic methods for production of Ampicillin, Amoxicillin and Cephalexin (none have yet been upscaled to industrial applicability) are the high costs (yield losses) and investments [illegible] when the amino .beta.-lactam [illegible] as raw material for the [illegible] .beta.-lactam. Furthermore, the starting [illegible] of the [illegible] are very low, typically less than [illegible] mM, [illegible] Ampicillin [illegible] enzymatic [illegible] of Ampicillin [illegible].

BSTB:
A process for enzymatic synthesis of Amoxicillin is described in Agric. Biol. Chem. 44 [illegible], [illegible] et seq., which process is performed in a reaction medium containing [illegible] volume/volume or more of [illegible]-propanol and [illegible] volume/volume [illegible]. When [illegible] of the last mentioned alcohols or 2.5 [illegible] of [illegible]-propanol [illegible], the initial concentration of the starting materials, D-.alpha.-(p-hydroxyphenyl)glycine methyl ester and 6-aminopenicillanic acid, is very low, i.e. [illegible] and [illegible] mM, respectively. When [illegible] of 2-propanol is used, the initial concentration of the starting materials, D-.alpha.-(p-hydroxyphenyl)glycine methyl ester and 6-aminopenicillanic acid, is 460 and 230 mM, respectively. It is stated in this paper that the addition of more than 100 mM of D-.alpha.-(p-hydroxyphenyl)glycine methyl ester and of more than 50 mM of 6-aminopenicillanic acid markedly suppressed the ration of conversion of 6-aminopenicillanic acid into amoxicillin. The conclusion of this statement is that this publication teaches away for increasing the [illegible] .beta.-lactam and of the acylating agent in the reaction mixture.

BSPR:
After the effective filing date of the application for a patent on this invention, namely Sep. 18-21, 1990, a poster was published at a NATO Workshop. The poster dealt with the preparation of cephalosporins and, according to this poster, working at low temperature had several positive effects on the [illegible]. The highest concentration of acylating agent used by this work was [illegible] mM of D-.alpha.-phenylglycine methyl ester and there was no indication [illegible] higher concentration of the acylating agent.

BSPR:
It has now, surprisingly, been found that the yield in the enzymatic preparation of .beta.-lactam derivatives can be improved by carrying out the reaction at high concentrations of the acylating agent.

BSTB:
By the process of this invention, it is possible and attractive to use [illegible] of the amino .beta.-lactam, for example 6-APA or 7-ADCA, without [illegible] of the amino .beta.-lactam, for example 6-APA or 7-ADCA, [illegible] purification [illegible] and investments in purification equipment for, for example, Ampicillin, Amoxicillin and Cephalexin are thus minimized as purification equipment formerly used for isolation of 6-APA [illegible] used for isolation of Ampicillin, Amoxicillin and Cephalexin.

BSTB:
[illegible] yield [illegible] this invention can be obtained [illegible] Ampicillin [illegible].

BSTB:

[illegible] a derivative of [illegible] or D-p-hydroxyphenylglycine.

DETD:
In another aspect, this process is characterized in that the concentration of the acylating agent, for example, the D-phenylglycine or D-p-hydroxyphenylglycine derivative, in the reaction mixture is above the solubility of said agent in the reaction mixture at least 10% above the solubility; or the initial amount of the acylating agent in the reaction mixture is about half or more than the initial amount of the amino .beta.-lactam in the reaction mixture, preferably above the solubility of the acylating agent in the reaction mixture the initial amount of the amino .beta.-lactam. In a still further aspect, this invention relates to a process whereby the concentration of acylating agent plus the concentration of .beta.-lactam derivative in the reaction mixture is above about 450 mM, preferably above about 500 mM, more preferred above about 650 mM, even more preferred above about 700 mM. In an additional aspect, this invention relates to a process whereby the initial concentration of acylating agent in the reaction mixture is above about 400 mM, preferably above about 500 mM, more preferred above about 600 mM, even more preferred above about 700 mM.

DETD:
Examples of .beta.-lactam derivatives that may be produced by the process of this invention are Ampicillin, Amoxicillin, Cefaclor, Cephalexin, Cephadroxil.

DETD:
The acylating agent may be a derivative of D-phenylglycine or D-p-hydroxyphenylglycine such as a lower alkyl ester, methyl, ethyl, n-propyl or isopropyl ester of an amide which is unsubstituted in the --CONH.sub.2 group. The acid or phenol group of the derivative may be used in the form of a salt, for example, the HCl salt of the H.sub.2 N.sub.2 salt. The acylating agent may be added in an active form or the active form may be formed in situ.

DETD:
The enzyme to be used in the process of this invention may be any enzyme catalyzing the reaction in question. Such enzymes [illegible] Enzymes to be used are, for example, Penicillin amidase or Penicillin acylase as classified as E.C. 3.5.1.11. A number of microbial [illegible] Such microorganisms, which in the examples hereinafter, [illegible] Escherichia, Bacillus, Proteus, Alcaligenes, Arthrobacter, Aeromonas, Xanthomonas, Kluyvera, Flavobacterium, Pseudomonas, Streptomyces, Achromobacter, Agrobacterium, Pseudomonas [illegible] European patent application having publication No. [illegible], Acetobacter pasteurianum, Bacillus megaterium, Xanthomonas citrii, European patent application having publication No. [illegible], Kluyvera citrophila Agr. Biol. Chem. 37 [illegible], [illegible] and Pseudomonas cepacia, European patent application having publication No. [illegible]. The Pseudomonas SY-77 enzyme is commercially available. The enzyme may be [illegible] penicillin hydrolase, acylase or amidase. In this [illegible], [illegible], [illegible], [illegible] [illegible], [illegible] [illegible].

DETD:

[illegible] enzyme [illegible] Immobilization may be [illegible] Immobilized [illegible] enzyme is commercially available from Boehringer Mannheim GmbH, Germany, under the trade name Enzygel.

DETD:
The solubility of the acylating agent such as the D-phenylglycine or D-p-hydroxyphenylglycine derivative will vary with the identity of the [illegible] solubility [illegible] However, the solubility is very dependent on the salt components in the solution, as well as on the pH value and the temperature of the solution. In some embodiments of the process of this invention, the initial reaction mixture is a slurry containing undissolved acylating agent and/or .beta.-lactam, which will dissolve partly or fully during the course of the reaction. The .beta.-lactam formed may precipitate during the reaction and, also, the hydrolysis products of the acylating agent such as D-phenylglycine and D-p-hydroxyphenylglycine, may precipitate. Hence, in many cases the reaction mixture will be a slurry throughout the reaction.

DETD:
The amino .beta.-lactam, for example 6-APA or 7-ADCA, used in the process of this invention may be obtained by enzymatic hydrolysis of the fermented penicillins or cephalosporins, (for example penicillin V, penicillin G or cephalosporin C) or their ring enlarged analogues (for example V-DCA and G-DCA) or derivatives thereof followed by removal of the hydrolysis by-product, if desired (phenoxyacetic acid etc.). Advantageously, the crude solution can be used directly without further purification or dilution.

DETD:
Generally, the reaction temperature of the process of this invention may vary between about [illegible].degree. C. and about 35.degree. C., is especially between about 10.degree. C. and about 30.degree. C. Temperatures in the range about [illegible].degree.-30.degree. C. may be preferred for convenient operation. The suitable pH value depends on the type and purity of enzyme. Using Escherichia coli enzyme, the pH value is typically in the range from about 5.5 through about 7.5, preferably in the range from about 6.0 through about 7. For the preparation of Amoxicillin, a pH value in the range from about 5.5 through about [illegible] is preferred. Control of the pH value may be used. Suitable reaction times are from several minutes to several hours, in particular from about [illegible] hour to about [illegible] hours. Suitable enzyme concentrations may be from about [illegible] ml to about [illegible] ml [illegible] enzyme activity, see below).

DETD:
Using the process according to this invention, extraordinary high yields can be obtained. The high yields are obtained using the teachings of this invention and properly selecting the concentration of the acylating agent, the ratio between the concentration of acylating agent and the starting amino .beta.-lactam, the pH value and the enzyme.

DETD:
[illegible] enzyme that hydrolyses per minute 1 [illegible] penicillin [illegible] penicillin G, [illegible] phosphate buffer, pH value [illegible], [illegible].degree. C.).

DETD:
[illegible] mM 6-APA and D-PGA [illegible] concentration as indicated in Table [illegible] pH value [illegible] and equilibrated at [illegible].degree. C. and [illegible]

DETD:

[illegible]

[0111]
[illegible] mM 6-APA and [illegible] mM D-PGM sulphate salt are adjusted to a pH value as indicated in Table 3, and the enzymatic synthesis is carried out at 37.0±0.5°C. [illegible] pH-stat titration, [illegible] ml and [illegible] Alcalase 2.5 DX type [illegible].

[0112]
[illegible] with 10 mM 6-APA and [illegible] mM D-PGM at pH value 6.4 and [illegible] of Alcalase 2.5 DX type from Novozymes with [illegible] ml and monitors the synthesis at temperatures as indicated in Table 4, the maximal yields of Ampicillin obtained are shown in Table 4.

[0113]
This example was performed analogously with Example 1 using D-PGM instead of D-PG. The maximum yields of Ampicillin obtained are as stated in Table 5.

[0114]
[illegible] penicillin from fermentation broth by filtration, extraction by [illegible] at an appropriate phase [illegible] as described in Table 1. [illegible] weight/volume) pen V is hydrolysed by Semacylase.TM. (immobilised pen V acylase from Novo Nordisk A/S) at a pH value of 7.6. The phenoxyacetic acid is removed by extraction and to the resulting 6-APA (150 mM) solution, containing minor amounts of biproducts from degraded pen V and 6-APA, is added 45 U/ml soluble enzyme from Escherichia coli and D-PGA to a final concentration of [illegible] mM. The pH value is adjusted to 6.4 and the reaction is allowed to proceed at 28.0±0.5°C, keeping the pH value constant.

[0115]
Under these conditions a total of 161 mmole of Ampicillin (MW) is formed per liter of reaction volume.

[0116]
[illegible] mg of immobilised enzyme is suspended in 10 ml with water. The enzyme solution was mixed with a solution of 6-APA and D-PGA to a total volume of [illegible] ml. The [illegible] 100 mM 6-APA and 75 mM D-PGA, having pH [illegible] and temperature 28°C. The synthesis [illegible] was [illegible] pH at 6.4 [illegible] 1 N sulphuric acid. [illegible] 6-APA was [illegible] Ampicillin.

[0117]
A mixture of 960 mg 6-APA and 3715 mg HPGA in water is adjusted to pH 6.2 at 15°C. 2, and 1250 U soluble penicillin G acylase from E. coli is added to a final volume of [illegible] ml. The synthesis is allowed to proceed at constant temperature, until [illegible] stop the pH at [illegible] with [illegible] 6-APA was [illegible] Ampicillin, corresponding to a yield [illegible] 6-APA consumed.

[0118]
[illegible] soluble penicillin G acylase from E. coli is added to a mixture of 6-APA and HPGA [illegible] mM and 75 mM final concentration, respectively, in water at pH [illegible]. After [illegible] the [illegible] keeping the temperature and pH constant using 1M sulfuric acid for the titration, 140 mM Amoxicillin was produced, yield based on HPLC-analysis.

[0119]
[illegible] with 10 mM 6-APA, 75 mM HPGA, 1250 U soluble penicillin G acylase from E. coli, [illegible] was produced after 4 hours, when the [illegible] pH 6.2 and 15°C.

[0120]
[illegible] as described in Example 4, using 100 mM 6-APA and 413 mM HPGA

...

[DEB]:
...-ADA is ...-aminoadipic acid, ...-AC is ...-aminocephalosporanic acid, ...-ADA is ...-phenylglycine, ...-PGM is D-phenylglycine methyl ester, ...-7ACA is ...-phenylglycinamido-7-aminocephalosporanic acid, 3-DCA is ...-phenylglycinamido-3-deacetoxycephalosporanic acid and HPGA is ...-p-hydroxyphenylglycine.

[DEC]:
...

[DED]:
Enzymatic synthesis of ...

[DEE]:
Enzymatic synthesis of ...

[DEF]:
Enzymatic synthesis of Amoxicillin.

DEFG:
Enzymatic synthesis of Amoxicillin.

DEFG':
Enzymatic synthesis of Amoxicillin.

[DEH]:
Retention times in minutes: 4.1 (D-PG); 6.3 (7-ACCA); 8.1 (6-APA); 9.1 (D-PGM); 10.1 (7-phenoxy); 13.4 (Amoxicillin); 18 (D-PGM).

[DEP]:
Retention times in minutes: 2.5 (D-p-hydroxyphenylglycine); 3.3 (HPGM); 5.4 (6-APA); 13.2 (Amoxicillin).

[DFI]:
...

TABLE 5 ... D-PGM, Amoxicillin formed, ... reaction time, mM ... hours

[DIB]:
... enzymatic preparation of a ...-lactam amide comprising ... a penicillin acylase from ... having sensitivity to a penicillin ... 7-amino-cephalosporanic acid, 7-amino-cephalosporanic acid and ... with an enzymatic ... in a pH ... than 7 ... volume system ... the ...-lactam amide is amoxicillin and the acyl ... is ...-alpha.-p-hydroxy-phenyl glycine methyl ester, the improvement ... sensitivity is eliminated ... penicillin acylase and the ...-lactam amide ... the system is maintained ... than 4.5 mM and the enzyme is derived ... a strain selected ... the group consisting of Escherichia coli, ... Bacillus megaterium, Kluyvera citrophila, Pseudomonas ...

[DIG]:
... Application ... Claim 1 ..., wherein the amide ...-lactam is ...-amino-...-thiazolyl-acetamido-3-cephem-4-carboxylic acid, 7-amino-3-deacetoxycephalosporanic acid,

[illegible]

TITL:
. A process according to claim 1 or 2, wherein the acylating agent is p-hydroxyphenylglycine or a derivative thereof.

TITL:
[illegible]

TITL:
14. A process according to claim 1 or 2, wherein the concentration of the amino .beta.-lactam in the reaction mixture when the enzymatic reaction starts is in the range from about [illegible] mM.

TITL:
15. A process according to claim 1 or 2, wherein the concentration of acylating agent in the reaction mixture when the enzymatic reaction starts is in the range from [illegible] mM.

TITL:
16. A process according to claim 14, wherein the concentration of the acylating agent in the reaction mixture when the enzymatic reaction starts is greater than [illegible] mM.

CLER:
17. A process according to claim 1 or 2, wherein the amount of the acylating agent in the reaction mixture when the enzymatic reaction starts is above the solubility of the agent in the reaction mixture.

CLER:
18. A process according to claim 1 or 2, wherein the amount of the acylating agent in the starting reaction mixture is greater than half the amount of said agent which is soluble in the reaction mixture plus the amount of the amino .beta.-lactam in the reaction mixture when the enzymatic reaction starts.

CLER:
19. A process according to claim 1 or 2, wherein the enzyme used is classified as EC [illegible].

TITL:
[illegible]

CLER:
20. A process according to claim 1 or 2, wherein an enzyme in reusable form is used.

CLER:
[illegible]